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NOTES ON THE TECHNIQUE OF HANDLING PREDACIOUS FUNGI

By C. L. DUDDINGTON

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Methods used in the study of predacious fungi include techniques for obtaining mixed cultures of the fungi from Nature, for isolating those that will grow in pure culture, for observing living material and for making permanent microscopical preparations.

CULTURAL METHODS

The last two decades have seen a great increase in our knowledge of the predacious fungi, most of which has come from the work of Drechsler, and they are beginning to interest a wider circle of mycologists, including university students. These fungi are easy to find and cultivate, but their peculiar mode of life makes it necessary to use certain practices—some might say malpractices—which depart more or less widely from orthodox mycological technique in order to bring them under observation.

Primary cultures

The best source of predacious fungi is rotting vegetable matter, preferably from on or near the soil. They are abundant in forest leaf-mould, and even more so in rotting bark and twigs and in pieces of decomposing leaf and stem from the floor of a wood or from a garden compost heap. To start cultures, about 2 g. of the material are placed in the centre of a plate of sterile agar and left to incubate at room temperature. The time taken for the fungi to appear may be anything from 1 week to 3 months, or longer; usually the mycelial Zoopagaceae preying on amoebae are the first to appear, followed by reticulate Hyphomycetes such as *Arthrobotrys oligospora*, while *Cochlonema* spp. and the ring-forming Hyphomycetes take longer to develop. The internal parasites of eelworms, such as *Harposporium anguillulae*, are usually late in appearing, partly because their early stages within the host are very difficult to detect. The cultures should not be kept at too high a temperature; different species of predacious fungi vary in their requirements (Drechsler, 1946, 1950), but 15–20° C. seems to be suitable for general work.

Inoculation of sterile agar with decaying organic matter naturally produces mixed cultures of fungi, bacteria, Protozoa, nematodes and—unfortunately—mites, together with occasional visitors such as moss protonemata, rotifers and even small annelids. Such a mixture is, up to a point, desirable and even essential in order to supply prey for the fungi, but the danger that the predacious fungi may be completely swamped by a heavy growth of moulds is obvious. To prevent this, a weak culture medium should be used.

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The most satisfactory one that I have yet found is maize-meal agar made up to the following formula: maize meal (or crushed maize grains), 20 g.; agar, 20 g.; tap water, 1000 ml. The maize and water are warmed to about 70° C. for 1 hr. After allowing time for the solid matter to settle, the supernatant fluid is filtered through glass wool and, after addition of the agar, autoclaved at 15 lb./sq.in. for 20 min. This medium permits the growth of enough bacteria to provide food for the Protozoa and eelworms that it is desired to encourage, but is too weak for vigorous development of the coarser moulds such as *Rhizopus*. Most predacious fungi grow well on it, even in pure culture.

Another useful medium is rabbit-dung agar. There are various formulae; one that I have found satisfactory is to steep rabbit pellets in tap water for 2 or 3 days, and pour off the supernatant fluid, filtering through glass wool if necessary; the liquor is then diluted with tap water until it is a pale straw colour and autoclaved after the addition of 2 % of agar. This medium encourages the multiplication of nematodes, but does not support good vegetative growth even of predacious fungi, which makes it useful for the study of internal parasites of nematodes or for material where, as sometimes happens, mould contamination is severe even on maize-meal agar. A disadvantage is that the agar often becomes rather soft, so that if eelworms are plentiful the surface may get badly churned up.

For soil predacious fungi an adaptation of the Warcup soil plate technique (Warcup, 1950; Duddington, 1954) is useful. About 2 g. of soil are scattered on the bottom of a sterile plate and sterile agar, cooled near to solidification, is then poured in, the soil being *slightly* mixed with the agar by gentle tilting of the plate. Mixing should not be too thorough, as if the soil is completely distributed over the plate subsequent observation is difficult. The agar used should be poor in nutriments, or mould contamination will be very severe; I generally use maize-meal agar with the maize liquor diluted with an equal volume of water before adding 2 % of agar. Unfortunately, spore production is often poor on this very weak medium, so that subculturing on something a little richer may be necessary before some of the predacious Hyphomycetes can be identified.

Subcultures

Many of the predacious fungi, especially some of the mycelial Zoopagaceae, are ephemeral in cultures, so that to keep them under observation for any length of time subculturing is necessary. This can be attempted by transferring a small block of agar bearing the fungus to a plate of sterile agar, taking care that some of the host animals are transferred at the same time. Even when this is done, the transfer often fails, and it is better if possible to transfer to a plate already containing plenty of the hosts. This is easy where the fungus attacks nematodes, as any plate containing eelworms will usually serve the purpose. A complication arises in dealing in this way with the Zoopagaceae, which are strongly host-specific (Drechsler, 1947), and the plate to which the transfer is made must contain the right species of animal. This may sometimes be arranged by transferring to a plate previously inoculated with some of the original material.

A more certain way of extending the life of a culture is by 'rejuvenation' of the agar surface. For this, sterile agar is cooled to a temperature just above the solidifying point, and a thin layer is then poured evenly over the surface of the old culture. The agar used should be very poor in nutriments to avoid overgrowth by moulds, and the medium that I have found best for this process is made by adding 2 % of agar to a very weak infusion of rabbit dung. The Protozoa and nematodes quickly come to the surface of a rejuvenated plate, followed by the fungi, and very fine material may often be obtained by this technique. If the plate is deep enough, the process can be repeated several times.

Plate cultures of predacious fungi often have to be under observation for a long time, and may become too dry. This danger can be minimized by keeping them in a closed tin or under a bell jar over water; the second method also helps to inhibit the spread of mites from batch to batch. It is useful to pour the plates while the agar is hot enough for condensation water to collect under the lids. Hot pouring is said to increase the chances of contamination owing to convection currents, but I am a little sceptical about this provided it is done carefully.

In setting up mixed cultures of predacious fungi, as described above, no adjustment of the pH of the medium seems to be necessary; for soil predacious fungi, in particular, the very acid media often used by soil mycologists should be avoided.

Pure cultures

The Zoopagaceae appear to be obligate predators and will not grow in the absence of their hosts. The endozoic Hyphomycetes such as *Harposporium* and its allies are as yet unrecorded in pure culture, but the reticulate and ring-forming Hyphomycetes of the genera *Arthrobotrys*, *Trichothecium*, *Dactylella* and *Dactylaria* are readily isolated and grow well on most of the usual media. These all produce sizable spores on long, erect fertile hyphae, and it is a simple matter to pick off spores on a particle of agar held on the end of a sterile needle, using a binocular dissecting microscope, and to transfer the spores to a sterile agar plate. This process is easiest if there are conidiophores of the fungus growing out horizontally from the small heap of material used to inoculate the original mixed culture, as the conidia will then be well above the agar surface, and their horizontal position makes it easy to see that the needle has touched nothing but the spores during the operation. Sometimes spore-picking is virtually impossible because the conidiophores of the predacious Hyphomycete are closely surrounded by some unwanted mould; if this is so, it may be advisable to cut out a block of agar about 5 mm. square from the place occupied by the fungus, for if the plate is then left for a few days it will often be found that conidiophores have grown horizontally into the space left by the removal of the agar block, so that spores can be picked off with great ease.

For growing most of the predacious Hyphomycetes in pure culture the maize-meal agar described above is excellent. Mycelial growth is thin, but sporulation is good and the fungi usually retain their predacious tendencies through many generations of subculturing—an important point to bear in mind in choosing a medium. Rich media (especially media with a high sugar content) should be avoided as, though the fungi may grow well, they often lose their aggressiveness. Some species sporulate very abundantly on 'Difco' bean pod agar, but this medium does not suit all. In maintaining stock cultures the interval between successive subcultures should not be too long, or the pathogenicity of the fungus may decline.

Most of the predacious Hyphomycetes do not form their characteristic traps in pure culture but do so in the presence of eelworms. To induce trap formation, it is better to add the fungus to a culture rich in eelworms rather than the converse, and the amount of inoculum added should be small. If too much agar is included in the transfer, the fungi may be slow in attacking the nematodes.

To obtain eelworm cultures, a strong infusion of rabbit dung is prepared by heating rabbit pellets in tap water. When the infusion is the colour of weak tea the fluid is decanted and, after the addition of 2 % of agar, autoclaved. Plates of this medium are inoculated with fresh horse or sheep dung or other material rich in eelworms, and usually after a few days nematodes cover the plates in enormous numbers. One of these plates is used to inoculate further plates of the same medium, the small pieces of agar inoculum being cut out from parts of the culture where microscopical observation shows that no predacious Hyphomycetes are present. These second series of plates are then used to inoculate plates of maize-meal agar or, preferably, rabbit-dung agar of normal strength, thus providing cultures of nematodes that should, in the course of a few days, be ready to receive the predacious fungi. I have not yet found a satisfactory means of maintaining stock cultures of free-living nematodes.

Observation of living material

Mixed cultures containing predacious fungi are ready for observation as soon as the initial growth of moulds, etc., has covered the surface of the agar completely. It is then possible to uncover the plates without serious contamination. Preliminary examination is best carried out with a $\frac{2}{3}$ in. objective, at a magnification of not less than sixty diameters to avoid overlooking the smaller Zoopagaceae. The presence of nematode-trapping Hyphomycetes is easily detected since the captured eelworms are usually conspicuous; the characteristic spores of these fungi on their long conidiophores also give warning of their presence, and if the surface of the agar shows nothing it is advisable to inspect the edges of the inoculum as both predacious Hyphomycetes and Zoopagaceae will often sporulate here before they appear on the agar. The mycelial Zoopagaceae are best recognized by their very fine hyphae with captured amoebae ranged alongside them, closer inspection with a 4 mm. objective revealing the haustoria of the fungi within the animals. Observation of the actual haustoria is essential to confirm that the mycelium is predacious, for amoebae will often disperse themselves along the hyphae of a harmless saprophyte—an irritating habit that can sometimes waste a great deal of time. Many of the Zoopagaceae only show good vegetative stages for a few days, so that if they are the object of study, cultures must be inspected at least twice a week, and oftener if possible. If Zoopagaceae are found it should be remembered that the form of their haustoria is of the greatest diagnostic importance; this should, if possible, be determined at once, and a small piece of material should be preserved in formalin-acetic acid for future reference. This excellent fixative and preservative consists of 10 parts of commercial formalin and 5 parts of glacial acetic acid in 85 parts of water.

If the vegetative stages of Zoopagaceae are not observed, the presence of these fungi will often be revealed by their spores. *Stylopage rhynchospora*, *S. rhicnacra* and *Acaulopage tetraceros*, for instance, are easily recognized by their conidia, and the filamentous spores of *A. baculispora* and its allies are very conspicuous, standing up like tiny bristles from the agar surface, often in large numbers. Even if no spores are seen with the low-power objective, it often pays to examine the surface with a higher power on the off-chance of observing fallen conidia or zygospores; the zygospores of Zoopagaceae are unmistakable.

Zoopagaceae internally parasitic in Protozoa are most easily detected by their conidia. The spore chains of *Cochlonema* spp. are particularly conspicuous. The endozoic Hyphomycetes such as *Harposporium* are also best found by looking for the fertile hyphae emerging from the bodies of dead eelworms, but these fungi present another problem as the infected nematodes often burrow to the bottom of the agar to die. Regular examination of inverted plates is therefore necessary, and if one of these endozoic Hyphomycetes is found its position can be marked by pressing the point of a fountain pen against the glass bottom of the plate so as to leave a small blob of ink. On holding the plate up to the light this can be seen beneath the agar, and a small block of agar bearing the specimen can be cut out.

Detailed examination of predacious fungi under the highest powers of the microscope should, wherever possible, be made with living material mounted in water, as stained preparations are far less satisfactory. A small block of agar is cut out and the surface bearing the fungus is sliced off with a razor blade and mounted on a cavity slide in a drop of water. Sometimes the structures to be examined are below the surface, and it may be difficult to use a 2 mm. immersion objective owing to the short working distance; to avoid this difficulty an apochromatic 3 mm. objective of high aperture is useful, as it will give a performance equal to that of the 2 mm. if used with a × 15 ocular. Such structures as the haustoria of Zoopagaceae are often very difficult to see, and the importance of first-class optical equipment and correct illumination can scarcely be exaggerated. Drechsler (1946) recommends the use of a high-power water-immersion objective for examining Zoopagaceae.

PERMANENT PREPARATIONS

Good permanent preparations of predacious fungi are not easy to make› and are less satisfactory to study than water mounts of living material. The endozoic Hyphomycetes are an exception, as they mount well in lactophenol cotton blue. This unfortunately gives poor results with the nematode-trapping Hyphomycetes and is useless for the Zoopagaceae. The latter can be mounted in cotton blue in lactic acid, but the preparations last for a few months at most.

Several techniques for making permanent mounts have been described elsewhere (Duddington & Dixon, 1951, 1952) and so will only be briefly outlined here. For the endozoic Hyphomycetes the erythrosin-glycerine method is useful. A small piece of agar containing the specimen is fixed in formalin-acetic acid for not less than 24 hr., and washed in several changes of water until no smell of fixative is detectable. The material is placed in a saturated aqueous solution of erythrosin for 24 hr., after which it is transferred to 10 % glycerine and allowed to concentrate until most of the water has evaporated; addition of 0·5 % of glacial acetic acid improves the staining. The dehydration is completed in a desiccator, and the material can then be mounted in glycerine or in glycerine jelly.

The nematode-trapping Hyphomycetes can be mounted satisfactorily in Venetian turpentine. Agar slices are fixed in formalin acetic acid, washed, stained in Delafield's haematoxylin for about 5 min. (the exact period depends on the material), 'blued' in tap water or ammonia vapour and placed in 10 % glycerine which is allowed to concentrate as in the erythrosin-glycerine method. When the concentration of the glycerine is complete, the material is washed in industrial methylated spirit until all glycerine is removed, and dehydrated in absolute alcohol. It is then placed in a 10 % solution of Venetian turpentine in absolute alcohol; this should be done in a desiccator since the dilute turpentine is very sensitive to moisture. The dish containing the material is left in the desiccator until the turpentine has concentrated to a suitable viscosity for mounting, the process ideally taking about 2 days. The preparations can then be mounted in Venetian turpentine, using a cavity slide. These preparations are beautifully transparent, in spite of the presence of agar.

If really thin preparations of nematode-trapping Hyphomycetes are required, agar blocks are fixed in formalin-acetic acid, washed and stuck fungus downwards with Szombathy's gelatine (Chamberlain, 1932) to a no. 1 cover-slip. The cover-slip is then put in a warm place (about 25–30° C. is recommended) until the agar begins to dry up and shrink. The surface bearing the fungus cannot shrink, as it is stuck to the glass. At a certain stage in the shrinkage, which is determined in the first instance by trial and error, the greater part of the agar is sliced away with a very sharp razor blade, leaving only a thin film stuck to the glass. This may then be treated in the same way as a microtome section on a slide, being stained with Heidenhain's haematoxylin, dehydrated with alcohol, cleared with xylol and mounted in Canada balsam. The dehydration and clearing must be carried out very gradually. Excellent preparations can be obtained by this technique, which is not as difficult as it sounds.

The Zoopagaceae are not good subjects for permanent mounts. Reasonably good preparations of spores can be made by the erythrosin-glycerine method, but one is lucky if the captured amoebae are not torn away from the mycelium during the manipulations. A modification of the chlorazol black technique of Armitage (1944) shows up the haustoria nicely, and has the advantage of rapidity. Agar slices bearing the fungus are fixed by dipping in absolute alcohol for 1 min. and then stained in a saturated solution of chlorazol black E in absolute methyl alcohol for from 1 to 3 sec.; they are then dehydrated in absolute alcohol for 10 min. and mounted direct in 'Euparal', using a cavity slide. Haustoria stain deeply by this method.

For the preservation of material, the formalin-acetic acid fixative is excellent. It causes no noticeable distortion, and the fungi can be left in it indefinitely without harm. I have a specimen of the very delicate *Stylopage haploe* Drechsler that has been kept in the fluid for 13 years and is still perfect.

IDENTIFICATION

Fortunately for the investigator, the literature on predacious fungi is reasonably compact, nearly all the known species having been described by Drechsler. The invaluable monograph by Dollfus (1946) contains the diagnostic characters of all nematode-attacking species up to the time of publication. For the Zoopagaceae the papers by Drechsler in *Mycologia* from 1935 onwards should be consulted.

There are some pitfalls. Some species appear to be rather labile, and the British strains often differ slightly from their American types, especially in the Zoopagaceae. *Acaulopage tetraceros* Drechsler and some of the species of *Zoopage* are examples of this. The genera *Dactylella* and *Dactylaria* are hard to separate; for instance, *D. psychrophila* Drechsler usually forms its spores singly, and could easily be mistaken for a *Dactylella* unless a fairly old pure culture is seen. In identifying the nematode-trapping Hyphomycetes the nature of the traps must be known; conidia by themselves are not sufficient. New species should be proposed with great caution.

In conclusion, I should like to express my thanks to Dr B. Barnes, whose suggestions and constant help over a period of many years led to the development of the techniques here described.

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A METHOD OF STUDYING ACTIVE MYCELIA ON LIVING ROOTS AND OTHER SURFACES IN THE SOIL

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(With 3 Text-figures)

A method by which the active mycelia on roots and other soil surfaces can be examined is described. It consists of serial washings in sterile distilled water, hence the degree of completeness of removal of spores can be checked by the dilution plate method. The method has been successfully applied to the study of fungal populations on the surfaces of beech mycorrhizas, root systems of seedling beech and the petioles of litter leaves and of leaves still attached to the plant. It is shown that the impression gained by studying populations growing on agar from unwashed surfaces is different from that obtained from washed surfaces because in the former sporing Hyphomycetes are greatly over-represented whilst Phycomycetes and more particularly slow-growing non-sporing mycelia are greatly under-represented. This method is being used in the study of the pattern of distribution of fungi on the root surfaces of seedlings and of pasture grasses.

Garrett (1951) recently discussed the characteristics of ecological groups of soil fungi and criticized methods of observing soil populations. The impression given by this and by other articles is that wide generalizations about the soil flora are unwarranted and that, at present, the results obtained from any series of observations must be stated strictly within the limits of accuracy and sensitivity of the methods used. All methods are in some degree selective even when the selection is determined only, or mainly, by the choice of a nutritive medium. It is no more possible to elaborate a single completely non-selective method for describing the flora of a given soil than it is to produce a single quantitative method for enumerating all species of green plants in a community. Indeed, even if such a method could be found, it would yield information of less interest and value than others by which conscious use of selection was used to describe the pattern arrangement of a soil flora.

One of the first requirements for an ecological study of soil organisms is that the methods must distinguish between organisms which are vegetatively active in the soil and play a part in the soil processes and those which exist in dormant or inactive form as spores and other propagules. The problem is one of great difficulty. Older methods of heat treatment and partial sterilization attempted to eliminate the vegetatively active mycelia in order to gain a knowledge of the importance of spores in dilution plate estimates of frequency (McLennan, 1928). Such methods are often subject to grave errors because the cultures obtained by dilution of soil may be so preponderantly from spores, that the estimates of vegetatively active forms are greatly affected by small variations in the number of spores. The dilution plate methods in all their variants give essentially a survey of actively sporing fungi and this is true even when such methods have been used selectively. For instance much of the existing work on rhizosphere fungi must be considered as providing information on sporing fungi only.

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The methods of Waksman (1916), Chesters (1940, 1948) and Warcup (1950) permit the identification of fungi selected and isolated in such a way that it is probable that they exist in the soil as active mycelia. The recent work of Dobbs & Hinson (1953) gives added justification for the belief in the effectiveness of methods which ensure that significant quantities of soil are present and in contact with the isolating medium. There is, nevertheless, no certainty that all the fungi isolated are actively mycelial in the soil samples used nor can it usually be ascertained to what microhabitat they belong.

The method described in this paper is a simple technique, which aims at obtaining information concerning the fungi which exist in active mycelial conditions on certain chosen soil sites. The conscious selection of defined kinds of substrate from the soil is the first stage. The repeated washing of the selected material ensures the detachment from it of spore and other propagules. The final stage of conscious selection may be applied by the presentation of any desired conditions of growth to organisms which remain as mycelia upon the washed material.

Methods similar to that to be described have been used by others (Kürbis, 1937; Simmonds, 1930; Simmonds & Ledingham, 1937; Robertson, 1954) for certain specific purposes. They consist of serial washings of selected material in repeated aliquots of sterile water. Here it is to be shown that provided the limitations of the technique are understood, the methods are widely applicable and can be used to gain valuable information concerning the ecology of soil fungi. For instance, the methods could be used as a second phase in the technique described by Chesters (1948), where soil material selected by washing is studied.

Plant material

The kinds of material on the surface of which the population of fungi was studied in the various experiments described below were as follows:

(*a*) Beech mycorrhizas from the litter horizon of the soil of a beech wood. These were obtained by removing the surface litter and collecting lateral root systems into sterile tubes.

(*b*) Petioles of the litter leaves from the same sites and depths as mycorrhizal roots and in close juxtaposition to them. Whole leaves were taken in sterile tubes to the laboratory and the petioles detached with a sterile scalpel.

(*c*) Whole root systems of beech seedlings, during their first year of growth. The seedlings were grown in selected soils in fibre pots, which could be removed to the laboratory and split by cutting to allow the removal of the root system with the minimum of damage.

(*d*) Petioles of the leaves of seedlings, picked before leaf fall.

APPARATUS AND MEDIA

All apparatus and media were sterilized by standard methods. Phials with screw-tops of 30 ml. capacity were employed for washing the samples of plant material. 5 or 10 ml. of sterile water were introduced into each phial, together with the plant material. These phials were shaken at about 300 beats per min. on a shaking platform, with an excursion of 4·0 cm.

When the fungal content of the water used for washing was investigated aliquots were transferred by means of sterile pipettes to tubes containing molten agar at 40° C., agitated by hand and poured into Petri dishes.

In each experiment the sterility of the apparatus, water and media was confirmed by suitable controls.

The following media were used: Czapek agar, Czapek peptone agar, glucose-peptone agar, Lochhead & Chase's (1943) non-selective medium (slightly modified), and Czapek-Dox as modified by Warcup (1950).

THE METHOD APPLIED TO ROOT APICES

Table 1 and Fig. 1 give the results of one series of experiments with beech mycorrhizas. The apical 2·0 cm. of mycorrhizal roots were transferred in batches of five or ten to sterile screw-topped phials containing 10 ml. of distilled water. The phials were shaken for periods of 2 min. on the shaker. At the end of each period the water was decanted into a sterile tube and replaced with a fresh aliquot and the phial shaken again. This process was repeated 30 times.

Table 1. *The decanting method of root washing*

(The mean number of fungi developing on dilution plates from 1 ml. aliquots of washes. Counts made on 4th day of incubation. Washing numbers indicate the number of changes of water made up to the sample. The symbol ∞ means an uncountable number observed.)

Date of sampling	1 Oct. 1951		8 Oct. 1951	
No. of roots per 10 ml. ...	5	5	10	10
No. of 1 ml. aliquots per washing	2	5	2	5
Washing number 1	∞	—	∞	—
2	17·0	—	132·0	—
3	5·0	—	43·0	—
5	0·0	—	22·5	—
10	1·0	—	10·5	6·4
15	0·5	1·0	10·5	3·8
20	1·0	0·0	2·5	1·4
25	1·0	0·0	2·0	2·8
30	0·0	0·2	0·5	2·6
Mean no. of fungi observed on root washed 30 times	0·6	0·4	0·6	0·9

1 ml. aliquots of certain of the washings were added to molten agar to test for fungal content. The cleaned roots after thirty washes were each placed in the centre of a Petri dish of nutrient agar.

It may be seen from Fig. 1 that the detachable fungal fragments capable of developing into a mycelium are mostly displaced in the first few washes, and thereafter the roots are gradually cleaned until a low, fairly constant number is displaced in each new aliquot after the twentieth washing.

During the process of decanting it is inevitable that some of the water used for washing will remain in the phial. It has been found that this does not exceed 0·5 ml. or 5 % of the volume used in this experiment. Hence, after two dilutions about 0·23 % of the original aliquot remains, but it contains a higher proportion of the heavier soil particles than that decanted.

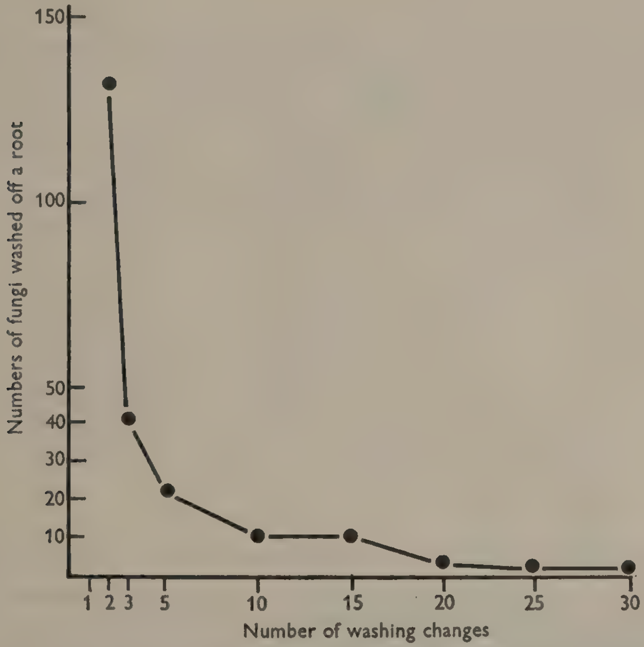

Fig. 1. Numbers of fungi washed off roots shaken in aliquots of sterile water.

When cleaning plant material of low specific gravity considerable quantities of detritus may settle in the bottom of the phial and not be decanted. In such cases it is especially important to transfer the plant material to a new phial after the first few washes. The technique of the removal of roots from phial to phial is slower than the decanting technique and is best carried out in a sterile room as it is more open to aerial contamination.

The degree to which roots may be cleaned of detachable propagules by these means is shown in Table 2 where a series of preliminary washings preceded a series of washings by decantation. In the final platings eight colonies were obtained on a total of thirty Petri dishes.

The final entry on Table 1 shows that roots cleaned by repeated washings have upon their surfaces fungi which will develop on suitable media. It is believed that these fungi exist there as hyphae and comprise that part of the true root surface flora which can develop in nutrient agar. Many samples of cleaned roots have been examined under the microscope after staining with cotton blue. The only soil particles found upon them were entangled in hyphae radiating out from the root surface. Spores were observed once, but intercellular swollen segments with thick walls, which appeared to be chlamydospores, were found. This visual observation confirms the view that the vast majority of colonies developing from material cleaned in the way described represent fungi having a vegetative existence on the living root surface.

Table 2. *A series of rough washes in sterile water followed by a series of washes by decantation may achieve satisfactory removal of detachable propagules*

(Experiment, 20 December 1946.)

Washing number and container	Description of washing	Description of washing
Petri dish 1	40 roots in 25 ml.	40 roots in 25 ml.
Shaken in phial A 2	40 roots in 10 ml.	10 roots in 15 ml.
B 3	40 roots in 10 ml.	10 roots in 15 ml.
Roots transferred to fresh phials C 4	5 roots in 5 ml.	5 roots in 5 ml.
5	5 roots in 5 ml.	5 roots in 5 ml.
6	5 roots in 5 ml.	5 roots in 5 ml.
7	5 roots in 5 ml.	5 roots in 5 ml.
8	5 roots in 5 ml.	5 roots in 5 ml.
9	5 roots in 5 ml.	5 roots in 5 ml.
10	5 roots in 5 ml.	5 roots in 5 ml.
11	—	5 roots in 5 ml.
12	—	5 roots in 5 ml.
Total colonies on 15 plates	8	0

THE METHOD APPLIED TO A COMPARISON OF DIFFERENT SURFACES

Table 3 gives the details and results of an experiment which compares populations obtained from the surfaces of mycorrhizal roots and the petioles of litter leaves collected from the same position in the soil. In order to reduce errors arising out of shape and size of the fragments of plant material used, the roots and petioles were chosen to have similar diameter (0·3 cm.) and length (1 cm.).

There is no doubt that the number of detachable propagules in the first washings of the petiole surfaces exceeded that on the living roots by a factor of nearly 10 during the first 3 days of incubation. In the subsequent 2 days the number of colonies developing from the petiole washings was so great that they interacted with one another on the plates and the counts must be considered as minimum estimates on those days.

After a period of incubation the clean plant material was examined microscopically and the types of colony developing from it were recorded. An average of 4·3 types of mycelia were recorded per cm. of root fragment and 3·0 types per cm. of petiole fragment. The minimal estimates of propagules obtained by washing were not connected with the diversity nor density of the surface mycelia. This important point is emphasized in section B of Table 3. The percentage incidence of three groupings of fungi in the washings and on the surfaces of roots and petioles are recorded. 'Penicillioids' include *Penicillium*, *Spicaria*, *Paecilomyces* and *Aspergillus*; 'Mucorales' include *Mucor*, *Zygorhynchus*, *Rhizopus* and *Mortierella*. In each case Penicillioids are greatly over-represented in the washings and the Mucorales and other fungi are under-represented. The high total numbers of colonies obtained from petiole washings are due mainly to a great spore production by Penicillia.

Table 3. *Comparison of numbers of detachable propagules upon the surfaces of 1 cm. length of beech mycorrhiza with similar lengths of beech petioles, together with estimates of active surface mycelia on the two types of material*

(Material collected from litter layer of Bagley Wood. Twelve petioles and twelve roots each washed in separate phials in 5 ml. of sterile water shaken for 10 min. Plant material was removed to new phials, cleaned with twelve further washes. Each fragment was then cut into four portions and plated on agar. Medium, acid Czapek, pH 4·2. Experiment, 2 July 1947.)

A. Comparison of total numbers of colonies developing from first washing of 5 ml. used for petiole or roots. 1 ml. used as inoculum

	Roots			Petioles		
Days of incubation...	3	4	5	3	4	5
Sample 1	87·5	122·5	130	1670	1912	—
2	90·0	115·0	122·5	420	983	—
3	57·5	90·0	105	507·5	1032	—
4	10·0	22·5	30	1525	1775	—
5	85·0	192·5	220	385	638	—
6	82·5	147·5	160	217·5	512	—
7	25·0	50·0	65	322·5	893	—
8	105·0	152·5	157·5	710	1085	—
9	77·5	130·0	197·5	822·5	980	—
10	122·5	297·5	345	387·5	600	—
11	37·5	67·5	112·5	475	735	—
12	35·0	67·5	92·5	660	828	—
Mean	67·9	121·3	144·5	675	998	—

B. Relative numbers of fungi (% of total colonies) developing in first washing and upon surface of clean roots and petioles

	Roots			Petioles		
	Penicillioids	Mucorales	Rest	Penicillioids	Mucorales	Rest
First washings	67	16	17	93	5	2
Surface (washed in 12 changes of water)	35	24	41	35	45	20

This result is an example of the application of the washing technique which gives information not only concerning the nature of the surface population but also concerning the sporing activity of the fungi present.

Tables 4 and 5 give an example which elaborates the points made by the last experiment. Tips of mycorrhizal roots and petioles 0·5 cm. in length were washed as before and the constitution of the population of recognizable mycelia was compared as follows: (*a*) unwashed samples, (*b*) first washings, (*c*) clean samples.

Table 4 gives a simple comparison of the estimated mean number of propagules in the first washing of root tips and petioles. In this experiment the mean surface areas of the plant segments used were estimated by micrometer measurements so that the number of organisms per unit surface area can be calculated.

These results are essentially similar to those of Table 3, and show that in this case the refinement of estimating the surface area of the samples does not materially alter the results. A vastly greater quantity of viable fungal propagules may be detached by a single washing from the same area of

Table 4. *A comparison of the mean numbers of fungal and bacterial propagules detached by the first washing from 0·5 cm. lengths of mycorrhizal roots and litter petioles of beech*

(Samples were taken from the litter layer of the soil in Bagley Wood. Czapek-Dox medium as modified by Warcup (1950). Incubated in dark at 25° C. Bacteria and Actinomycetes counted after 3 days' and fungi after 4 days' incubation. Experiment, 11 August 1953.)

	No. of colonies per segment		No. of colonies per mm.^{2}		
	Fungi	Bacteria and Actinomycetes	Mean surface area mm.^{2}	Fungi	Bacteria and Actinomycetes
Mycorrhizal root tips	66	1610	14·3	4·6	112
Beech litter petioles	776	1843	20·3	38·1	91

Table 5. *Mycorrhizal roots and petioles collected from the litter layer of Bagley Wood*

(Material cut into 0·5 cm. lengths. Samples of ten roots and petioles were plated unwashed, and some after twenty washing changes. First wash sampled by dilution plate method. 1·0 ml. taken from 10·0 ml. of washing water. Medium Czapek-Dox (Warcup, 1950). Incubated at 25° C. in the dark for 9 days. Results are expressed as percentage occurrence on plates or as a percentage of total colonies. Experiment, 11 August 1952.)

	Petioles			Roots		
	Dilution plates	Unwashed	Washed	Dilution plates	Unwashed	Washed
Subjective estimate of fungal growth after 3 days	—	64	40	—	20	4
Composition of fungal flora	% colonies	% occurrence		% colonies	% occurrence	
Sterile mycelia	1·0	3·1	6·9	4·6	12·0	54·6
Phycomycetes						
Mucor spp. (*M. hiemalis*)	—	21·9	13·8	—	12·0	9·1
Mucor sp. with zygotes	—	12·5	17·2	—	4·0	—
Mucor ramannianus	—	12·5	17·2	1·5	4·0	9·1
Mortierella spp.	—	—	—	7·7	12·0	—
Hyphomycetes						
Penicillium spp.	12·2	12·5	6·9	38·5	24·0	9·1
Pullularia sp.	0·6	3·1	10·4	—	—	—
Cladosporium sp.	85·1	6·3	—	10·8	—	—
Fusarium spp.	—	3·1	13·8	—	—	—
Hyalopus spp.	—	12·5	10·4	1·5	24·0	—
Trichoderma viride	0·1	9·4	3·5	—	8·0	—
Stemphylium sp.	—	3·1	—	1·5	—	—
Monilia geophila	—	—	—	27·7	—	—
Oospora sp.	—	—	—	—	—	9·1
Cylindrocarpon spp.	—	—	—	—	—	9·1
Other sporing spp.	0·8	—	—	6·4	—	—
No. of colonies examined	776	32	29	27	27	11

litter petioles than from the roots. There is very little difference in the numbers of viable bacteria and Actinomycetes detached in this way.

Table 5 gives an account of the proportion of various categories of fungi observed in the populations developing from unwashed and clean petiole and root fragments and in plates made from their washings. It illustrates clearly that the impression of the population of surface mycelia obtained by the washing technique differs from that obtained by the use of dilution plates or unwashed fragments. Phycomycetes were greatly under-represented in the washings, but some of the sporing Hyphomycete species were over-represented. The sterile mycelia, that is forms which fail to fructify in the culture media used, were, as might be expected, also greatly under-represented in the dilution plates. The table also shows that the two kinds of plant material were conducive to the growth of strikingly different populations, especially when judged by the active mycelia present on their surfaces, i.e. by comparison with fully cleansed material.

The fourth line in Table 5 gives entries under the title of 'Subjective estimate of fungal growth after 3 days'. The figures here were obtained by scoring each segment examined for the quantity of mycelium emanating from it after the time stated. The actual method of scoring may be modified to suit the information desired. In this case, each segment producing a mycelial mat greater than 1 cm. in diameter was scored 2, less than 1 cm. was scored 1 and no growth was scored 0. The mean score for any treatment obtained in this way gives an estimate of the rate of growth of the fungal population upon the medium used. It is clear from the figures that the washing technique removes a part of the population which is rapid in the rate of spread. This part is indeed made up of the rapidly growing, sporing fungi such as *Penicillium* spp., *Trichoderma viride*, *Cladosporium* spp. and it is the absence of the spores of these that allows the slower growing, delayed sporing types, here listed as sterile mycelia, to develop. This group contained, in this particular example, not only mycelia which have never been seen to fruit during the course of this work, but also forms of *Fusarium*, *Cylindrocarpon* and *Gilomastix* for instance which will develop fruiting structures in time.

Table 6. *A comparison of the mean numbers of fungal and bacterial propagules detached by the first washings from 0·5 cm. lengths of root tips and living petioles of beech seedlings from the experimental garden, Oxford*

(Medium Czapek-Dox as modified by Warcup (1950). Incubated at 25° C. Bacteria and Actinomycetes counted after 3 days, fungi after 4 days. Experiment, 8 August 1953.)

	No. of colonies per segment			No. of colonies per mm.²	
	Fungi	Bacteria and Actinomycetes	Mean surface area mm.²	Fungi	Bacteria and Actinomycetes
Seedling root tips	34	1529	10·1	3·4	151
Seedling petioles	492	6	18·2	27	0·3

A comparison of a similar kind using different material is given in Tables 6 and 7. Here the apical 0·5 cm. of young lateral roots of beech seedlings grown in garden soil, are compared with similar lengths of living petioles, picked from the shoots of the same plants. Here again the sterile mycelia and Phycomycetes are greatly under-estimated by the dilution plates and the Hyphomycetes over-estimated. The striking result is obtained that the petioles, especially the cleaned samples, possess a larger and more diverse surface flora than the root apices. No general conclusion must be drawn from this, because not only will the surface flora of the petioles change with age and condition, but also, as will be seen in a later section, active root apices are the least colonized part of the root surface of young seedlings.

Once again the subjective estimate of growth (Table 7) made in the same way as before shows that the rapidly spreading fungi are greatly over-estimated in the unwashed root. The petiole population, on the other hand, is constituted of a larger population of rapidly spreading and sporing forms for the subjective estimate is much less changed by washing.

Table 7. *Roots and petioles from 2-year-old seedlings growing in nursery garden soil*

(Material cut into 0·5 cm. lengths. Ten roots and petioles plated, unwashed, some plated after twenty washing changes. First washing sampled by dilution plate method, 1·0 ml. taken from 10·0 ml. of washing water. Medium Czapek-Dox (Warcup, 1950). Incubated at 25° C. in the dark for 9 days. Results expressed as percentage occurrence or as a percentage of colonies. Experiment, 8 August 1953.)

	Petioles			Roots		
	Dilution plates	Unwashed	Washed	Dilution plates	Unwashed	Washed
Subjective estimate of fungal growth after 3 days	—	100	75	—	65	15
Composition of fungal flora	% colonies	% occurrence		% colonies	% occurrence	
Sterile mycelia	2·2	9·3	24·5	0·0	0·0	45·5
Phycomycetes						
Mucor hiemalis	—	2·3	—	—	8·3	—
Mortierella sp.	—	—	—	8·3	16·7	27·2
Hyphomycetes						
Pullularia spp.	70·4	23·2	22·2	—	—	—
Alternaria sp.	0·8	20·9	20·0	—	—	—
Cladosporium sp.	24·4	20·9	17·8	5·6	—	—
Botrytis sp.	1·4	14·0	13·4	—	—	—
Fusarium spp.	0·6	7·0	2·2	2·8	33·1	9·1
Stemphylium sp.	—	2·3	—	2·8	—	—
Penicillium spp.	0·2	—	—	72·2	16·7	18·2
Trichoderma viride	—	—	—	2·8	16·7	—
Other sporing fungi	0·2	—	—	5·6	8·3	—
No. of colonies examined	492	43	45	34	12	11
Total	100·2	99·9	100·1	100·1	99·8	100·0

Two points of general importance have been illustrated by the experiments of this section. The colonies obtained in the first washings of the roots are exactly those which would be observed in a study of the rhizosphere by the dilution plate method. They arise from two sources—from spores and mycelial fragments which exist in the rhizosphere soil and from spores of the root surface fungi which are vegetatively and reproductively active on the root surface. Secondly, the later washings contain the 'root surface fungi' as defined by Webley, Eastwood & Gimmingham (1952), and of course may well include a higher proportion of true root surface colonists. Nevertheless, the proportions of true root surface forms which are detached during the secondary washings will be affected by the nature of previous treatment—for example the volume of washing water and the speed of shaking. It seems doubtful, perhaps, whether the study of these mixed populations can be very valuable because they are derived from several sites in the root region. Only those mycelia observed to grow from cleaned root surfaces can really be accepted as true root surface colonists.

THE METHOD APPLIED TO ROOT SYSTEMS

The method of washing roots

The method used above for obtaining vegetatively active surface mycelia on apical portions of roots or on leaf fragments can be used for investigating root systems of seedlings of trees or herbaceous plants. It has been applied with some success to the study of the seedlings of *Fagus sylvatica* and to roots of grasses—detailed accounts of which will be published later.

Seedlings were removed from the fibre pots by carefully cutting away the fibre, were gently shaken free of soil, the root system placed in sterile boiling-tubes with the stem and leaves protruding through the cotton-wool plug and the tops were cut off 1 cm. above the soil level. Each root system was transferred to a sterile dish of distilled water and gently freed of soil particles. As the water became cloudy the roots were transferred to another dish and this process of gentle preliminary washing was repeated until the system appeared clean. During this process unwanted parts of the system were removed.

The appropriate part of the system was cut into 5 cm. lengths and one of these placed in each labelled washing phial and cleaned with repeated changes of water as described above. After twenty changes the 5 cm. lengths were cut into equal segments (0·2 cm. segments were found to be convenient in size) in Petri dishes. As each small segment was cut it was lifted on the tip of a scalpel on to a plate of nutrient agar and gently pressed into the medium. The plates were labelled so that the position of each segment on the root system was recorded. It was possible to space five to ten segments upon one nutrient agar plate to economize in medium and time.

In the examples quoted below the medium used was slightly modified from that described by Lochhead & Chase (1943) containing glucose, salts, amino-acids and growth factors. The cultures were incubated at 25° C. for 3 days and thereafter at room temperature for a further period.

The methods of recording results

The scoring of cultures made in this way depends upon the nature of the information required. Direct inspection and subjective scoring of the plates after 3 days at 25° C. is used to determine something of the nature and mode of growth of the surface flora. In Fig. 2 the proportion of segments from which hyphal growth visible with a magnification of 10 × after 3 days is plotted against the distance from the tap root apex for two samples of four beech seedlings 45 and 59 days after sowing. A majority of the segments near the apex appear to be sterile but the more proximal regions of the roots bear rapidly growing fungi. The picture of the root surface population obtained in this way is not a simple one, for in later counts some of the sterile segments eventually produce slow-growing mycelia. Hence in the early stages of seedling growth it appears that the rapidly growing apical region of the tap roots is not only sparsely colonized by mycelia but is also deficient in rapidly growing forms.

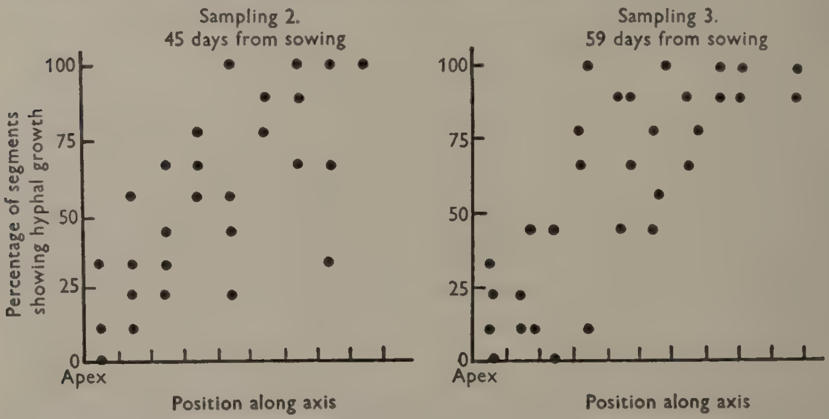

Fig. 2. The proportion of root segments showing hyphal growth plotted against distance from the root apex.

Table 8. *The percentage of non-sporing mycelia obtained by microscopic observations and isolation from the root surface of beech seedlings*

(Apical parts and proximal parts of the tap roots are compared.)

Date from sowing	45 days	59 days
Apical 8 cm.	53	58
Remainder	16	25

In the final counts of these two sets of samples (Table 8) the mycelial isolates were classified under two heads, sporing and non-sporing forms.

It is clear from these data that the number of slow-growing fungi is likely to be under-estimated on the older parts of the root as the fast-growing sporing fungi may overgrow and obscure them.

Further information may be obtained by the recognition of the types of fungi concerned. After a series of preliminary experiments a majority of the mycelial and hyphal types can be recognized and given a code number even if they cannot be named. Fig. 3 illustrates one example of the conclusions reached concerning the distribution of mycelia upon a single lateral root of beech. The sample was taken in October from a first-year seedling grown in a garden soil. The number of kinds of fungi per centimetre of root increased from apex to base, and it is possible that the order in which the kinds occur is partly the result of the order of colonization of the root as it ages. *Mycelium radicis atrovirens* at the tip is succeeded by *Hyalopus* and *Cylindrocarpon* followed by a black mycelium forming sclerotia and finally by *Rhizoctonia* (*sylvestris*?). Underlying the last is an unidentifiable mycelium completely overgrown. The diagram also shows that the sublaterals may be in part colonized proximally from the mother root. A large proportion of the surface fungi in this example remained

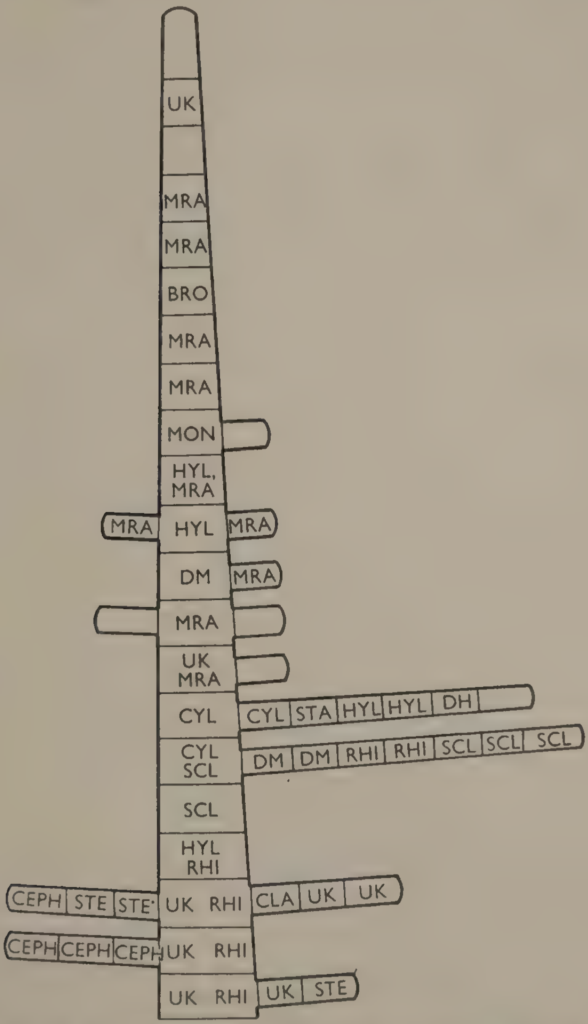

Fig. 3. The distribution of fungi on the surfaces of a lateral root system of a beech seedling grown in a nursery soil. UK, unknown mycelium; MRA, *Mycelium radicis atrovirens*; BRO, brown sterile hyphae; MON, *Monilia* sp.; HYL, *Hyalopus* sp.; DM, dark sterile mycelium; CYL, *Cylindrocarpon* sp.; RHI, *Rhizoctonia* sp.; SCL, sclerotial primary fungus with dark mycelium; CEPH, *Cephalosporium* sp.; CLA, mycelium with clamps; STE, sterile hyphae; STA, *Stachybotrys* sp.; DH, dark mycelium with sparse growth.

sterile in culture. This has been found to be generally true of the apices of tap roots and of lateral roots.

An extension of such observations may be made on the lines shown in Table 9. Here a comparison is made between the fungal populations of tap roots of beech seedlings grown in three different soils. Certain of the more frequently occurring isolates were grown on the sampling medium used to determine their relative rates of lateral spread in culture.

Table 9. *A comparison of the surface fungi of the tap roots of beech seedlings grown in three different soils*

(The 0·2 cm. segments were classified according to the extent of mycelial development from them after 3 days' incubation. The frequency of certain species where growth rate in culture has been determined is shown in each of the different subjective groups and is recorded as a mean per 100 segments of root.)

	Beechwood soils						Nursery garden soil		
	Shotridge			Bagley					
Subjective grouping ...	0	1	2	0	1	2	0	1	2
Slow-growing fungi									
Sterile hyaline	2	11	n	11	n	4	12	3	2
Sterile dark	0	n	0	0	n	0	7	1	1
Monilia geophila	7	2	n	4	n	3	3	1	n
Calcariosporium sp.	7	1	n	4	5	1	1	1	1
Verticillium sp.	9	3	2	1	1	1	1	1	n
Fungi of medium growth rate									
Cylindrocarpon sp.	32	43	26	40	35	15	18	34	18
Penicillium spp.	2	6	8	23	37	33	20	26	19
Fungi of fast growth rate									
Mortierella spp.	27	28	32	3	8	17	3	3	4
Mucor ramannianus	4	9	14	n	3	7	0	0	n
Oospora sp.	2	4	7	2	2	4	4	2	1
Trichoderma viride	4	2	5	2	2	6	0	n	6
Mucor spp.	0	n	2	n	1	9	0	0	n
Gliocladium roseum	0	n	n	1	2	0	5	5	6
Fusarium spp.	0	0	0	0	0	0	2	1	32
Trichocladium opacum	0	0	0	0	0	0	7	6	9

n indicates frequency less than 2.

It can be seen that in general the slow-growing species are more abundant on the segments of low subjective index and the fast-growing species on segments of index 2, although the correlation is by no means perfect. Hence one may conclude that the variation of subjective index is in the main due to pattern arrangement of species of differing growth rate, not to a variation in the condition of species uniformly distributed. Interaction and competition, as well as variation in the nature of the root surface, no doubt occur and may account for the occasional presence of potentially fast-growing forms on segments which show only slow development of a hyphal mat. The distribution of fungi upon the root surface can therefore be explained by variations in that surface and variation in the surrounding soil in respect of moisture, aeration, etc.

These results demonstrate that the differential growth rates of mixed populations of fungi on nutrient agar may lead to an under-estimate of the slow-growing fungi especially where large numbers of fast-growing forms occur. This source of error is affected by variations in the size of the inoculum, for the fast-growing fungi may be favoured on segments of large size. The consideration of this complicating factor will be dealt with later.

CONCLUSIONS

The method here described combines methods used by Kürbis (1937), Glynne (1939), Simmonds & Ledingham (1937) and many others. Robertson (1954) has made use of a more severe washing technique with unsterile tap water which has yielded similar results. All these are attempts to meet the common demand to know which of the fungi appearing in culture are derived from spores and which from mycelia. Using the present method certain results stand out clearly. First, that the majority of fungi described in rhizosphere studies are derived from spores produced by mycelia in the soil, rhizosphere and root surface regions. Secondly, that a sharp contrast exists between the populations of living mycelia on root surfaces and those on other soil surfaces. This contrast is most emphatically demonstrated when surfaces cleansed of spores are compared, i.e. when vegetatively active mycelia are compared. Thirdly, that on living root systems many slow-growing sterile mycelia and mycelia slow to produce spores occur which are not seen when unwashed root surfaces or dilutions of washings from root surfaces are cultured. These slow-growing forms comprise a considerable proportion of the mycelia present upon the apical actively growing parts of roots, but although they may also be present on the older regions of roots they are not dominant in the surface population. The absence of most of these mycelia from the surfaces of litter fragments suggests that they belong perhaps to the group of 'root-inhabiting' fungi which have been distinguished from the actively saprophytic and rapidly growing 'soil-inhabiting' fungi by Garrett (1951). Use of selective media should enable a more complete isolation and analysis of the activities and distribution of such fungi to be made.

Modifications of the method have shown it to be suitable to various problems. Interesting results have been obtained in the study of the pattern arrangement of fungi upon the surfaces of root systems of seedlings and upon the succession of fungi on the surfaces of grass roots in pasture.

Complexities arise when comparisons are made of the fungal populations of samples of plant material of widely different surface area, and these will be considered in detail later.

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A QUANTITATIVE STUDY OF AIR-BORNE FUNGUS SPORES IN DUNEDIN, NEW ZEALAND

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(With 1 Text-figure)

By means of culture plates exposed in a Manning slit sampler, a quantitative survey of air-borne moulds has been made between March 1953 and April 1954 in and just outside a building in the city area of Dunedin, New Zealand. There was little difference between the yields from indoor and outdoor air. The average count throughout the year was 13·9 colonies per cubic foot. Lowest yields were obtained during the winter months (an average of 5·9 colonies per cu.ft.) and highest during the summer (25·1 colonies per cu.ft.). *Cladosporium* occurred most frequently (42·9 %), and *Penicillium* was the next most commonly encountered mould (34·2 %).

Air-borne yeasts in the Dunedin city area were also surveyed between February 1953 and April 1954 by means of culture plates exposed in a slit sampler. The average density of viable yeasts was found to be one to two cells or clumps of cells to 2 cu.ft. of air. Almost all the isolates belonged to four major groups, *Debaryomyces* species (including imperfect forms of this genus), non-pathogenic *Cryptococcus* species, the *Sporobolomyces–Rhodotorula* group, and the yeast-like phase of *Cladosporium*. *Debaryomyces kloeckeri* and *Cryptococcus diffluens* were the species most commonly isolated.

Experiments have suggested that air-borne yeasts may be selected by their ability to withstand the effects of drying and ultra-violet radiation.

I. MOULDS

Mould surveys of the air for estimating the amount of fungal allergen present are usually made by one or both of two methods. The exposure of culture plates gives information upon the numbers and nature of spores of those moulds which can be cultivated, and the exposure of sticky slides enables counts to be made of the entire spore load, but gives an incomplete picture of the types comprising it. Dye & Vernon (1952) have made surveys of air-borne moulds in New Zealand by the first method, one of their sampling stations being in the Dunedin area. They exposed their plates for 5 min. on stands well away from buildings and other obstructions.

The purpose of the work described in this paper was to determine quantitatively the aerial spore load throughout the year in the Dunedin city area by means of culture plates in a Manning slit sampler. This method has the advantage of defining the spore load in absolute figures which are independent of such factors as wind and settling rate. The method is a simple one and avoids the errors inherent in older procedures where aliquot samples of air are bubbled through broth which is plated out. Measurable samples of air are drawn through a fine slit in a stream which impinges on a slowly revolving plate of culture medium. Spores stick upon the damp surface of the medium; the plate is then removed and incubated.

Two sampling sites were used. The first was a laboratory in a part of the Medical School, University of Otago ('indoor site'), which was centrally heated but not air conditioned, ventilation indeed was rather poor. The second was a studio on the roof of the School ('outdoor site'), also without air conditioning. The sampler was placed on the sill of an open window. This site was used rather than one in the open where rain might have interfered with sampling. Collections were made at 1 p.m., first at the indoor site and immediately afterwards at the outdoor one. Samples were taken on seventeen occasions between March 1953 and April 1954, mainly at four-weekly intervals. Fortnightly sampling was attempted at first, but because of pressure of other work it was not possible to maintain this.

Plates (100 mm. diameter) were exposed in a Manning slit sampler which could be adjusted to an intake of $\frac{1}{2}$, 1, 2 or 5 cu.ft. of air. The medium used was Sabouraud agar which had been adjusted to pH 4 by the addition of 1 N-HCl immediately before pouring the plates. This medium has been found to inhibit actinomycetes and air-borne bacteria, but not to interfere with the growth of fungi. Plates were poured immediately before use to ensure a uniform stickiness of surface. Five plates per site were exposed to 1 cu.ft. of air each on most occasions, but once or twice 2 cu.ft. were sampled. This was not very satisfactory, for although plates were often sparsely seeded it was impossible to predict accurately when this would happen. Counts from these larger samples have been adjusted to the usual figure of 1 cu.ft. of air per plate in the figures given under 'Results'. Plates were incubated at room temperature for 4 or 5 days before examination. As many colonies as possible were identified *in situ*. Those which could not be identified at this age were subcultured to Sabouraud agar slopes (pH 5·5), kept for periods of up to 3 months, and periodically examined. Yeasts were subcultured into dextrose-yeast extract broth; a detailed account of these is given in section II of this paper.

RESULTS

A total of 2373 colonies, 2244 of which were moulds and 129 yeasts, were isolated from 170 cu.ft. of air. No bacteria or actinomycetes grew on the medium. The difference in number of isolates from the two sampling sites was not statistically significant ($P > 0·82$). Taking the readings from both sites together, the average yield throughout the year was 13·9 colonies per cu.ft. Lowest yields were obtained in the period August to October (average: 5·9 colonies per cu.ft. of air), and highest in the January to March period (average: 25·1 colonies per cu.ft.). The summer rise in numbers at the indoor site was not significant ($P > 0·24$ for the November–April period compared with the May–October one), but the rise at the outdoor site was ($P = 0·005$). The *Penicillium* shower in June, the effects of which happened to be more pronounced indoors than outdoors, may have been responsible for the lack of a significant winter drop at the indoor site. The results of the monthly collections are summarized in Fig. 1 and Tables 1 and 2.

The dominant mould genus was *Cladosporium* which made up 42·9% (962 isolates) of the total mould isolates, and the next most frequent was *Penicillium* with 34·2% (767 isolates). Other genera occurred only sporadically. Many of the isolates labelled 'Mycelia Sterilia' would, on appropriate media, probably have produced spores. A number of

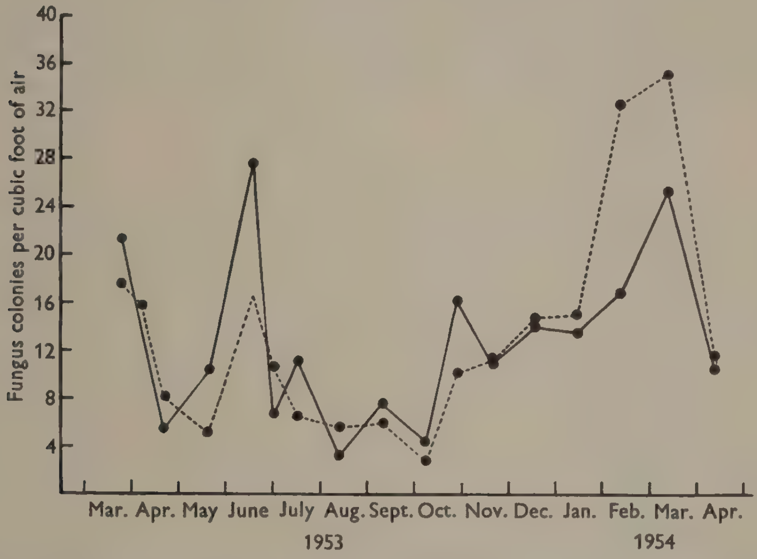

Fig. 1. Monthly fluctuation in spore content of air. Continuous line: counts at indoor site; broken line: counts at outdoor site.

Table 1. *Counts of total and predominant moulds throughout the sampling period. Samples are of 5 cu.ft. of air*

	Indoor site			Outdoor site		
Date	Total moulds	*Penicillium*	*Cladosporium*	Total moulds	*Penicillium*	*Cladosporium*
25. iii. 53	107	50	50	88	28	54
7. iv. 53	68	4	30	79	25	16
22. iv. 53	28	5	4	41	8	6
20. v. 53	52	36	14	26	14	9
17. vi. 53	139	135	1	83	45	2
1. vii. 53	33	26	4	53	34	3
15. vii. 53	56	28	22	33	19	7
12. viii. 53	16	7	5	28	6	14
9. ix. 53	38	16	10	30	21	3
7. x. 53	22	8	5	14	2	2
28. x. 53	31	43	10	51	25	11
25. xi. 53	55	13	16	56	5	16
16. xii. 53	70	6	56	74	17	48
13. i. 54	68	7	28	75	10	40
10. ii. 54	84	12	64	173	39	120
10. iii. 54	127	11	104	186	27	139
7. iv. 54	52	22	19	58	13	29
	1096	429	442	1148	338	520

isolates appeared to be *Rhizoctonia* species. Rather more isolates of *Cladosporium* and sterile mycelium and fewer of *Penicillium* were recovered from the outdoor site than from the indoor one.

Table 2. *Moulds isolated from* 170 *cu.ft. of air at Dunedin*

	Indoor site	Outdoor site	Total
Cladosporium	442	520	962
Penicillium	429	338	767
Sterile mycelium	115	165	270
Aspergillus	18	15	33
Sporotrichum	10	18	28
Botrytis	10	13	23
Trichoderma	18	5	23
Geomyces	8	8	16
Pycnidial types	6	4	10
Mucorales	5	5	10
Verticillium	3	7	10
Alternaria	2	6	8
Geotrichum	1	5	6
Oospora	2	4	6
Sclerotium	3	3	6
Pullularia	1	3	4
Cephalosporium	3	.	3
Scopulariopsis	3	.	3
Stemphylium	2	.	2
Monosporium	1	1	2
Spicaria	1	.	1
Epicoccum	.	1	1
Fusidium	.	1	1
Helminthosporium	.	1	1
Periconia	.	1	1
Monilia	.	1	1
Unidentified	5	8	13
Overgrown or failed to grow on subculture	8	14	22
	1096	1148	2244

Day to day variation

It is well known that abrupt changes in spore concentration may occur daily or even hourly (Ainsworth, 1952*a*, p. 76). To gauge the reliability of the discontinuous samplings made here, a series of daily counts was made for 8 days following the regular monthly collection of 7 April 1954. One plate was inoculated with 1 cu.ft. of air at the indoor site at 1 p.m. on each of these days. The average number of colonies per plate at this site on 7 April 1954 was 11·6, the average number during the control period was 12·6. Statistical analysis shows that 87·5 % of the observations made in the period 8–15 April 1954 fell within the 95 % confidence limits of the regular monthly collection of 7 April.

Discussion

Size of inocula

During spore showers of *Cladosporium* and *Penicillium*, as Dye & Vernon (1952) have pointed out, there is such crowding of plates that not only must all slower growing fungi be suppressed, but also many members of the dominant genus be unable to develop. Even in a relatively sparsely seeded culture it appears that very slow growing species have been overrun by more vigorous ones. Ingold (1953, p. 101) states: 'It is noteworthy that none of the usual methods normally reveals the presence of the basidiospores of the larger fungi in the air. They grow too slowly to be recorded in the exposed Petri-dish method.' The mortality of all moulds must be considerable when a colony of a rapidly growing type, such as *Rhizopus* or *Trichoderma* is present. An inoculum of 0·2 or even 0·1 cu.ft. of air per plate, whilst probably resulting in a number of sterile cultures during the winter months, would no doubt give greater counts per cubic foot than the larger inocula used in this work.

Sampling error

Whilst sixty-five yeasts were isolated from 85 cu.ft. of air from the indoor site when 1 cu.ft. of air was inoculated per plate, it was found during the sampling for yeasts (see Section II), when 5 cu.ft. of air were sampled per plate under similar conditions, that only 418 yeasts were recovered from 850 cu.ft. of air. It was considered that much of this reduction was due to overcrowding and suppression of colonies on the more heavily inoculated plates; also that some discrepancy in count might be due to the fact that collections for the yeast survey were made from 10.30 a.m. onwards, and for the mould survey at 1 p.m. However, there might also have been an error in count due to an initial contamination of plates as they were opened and placed in the sampling chamber. A check was made by inoculating a plate in the usual way with 1 cu.ft. of air and then treating a second plate in exactly the same manner but not drawing air through the sampling chamber. After incubation eighteen mould colonies developed on the inoculated plate and the control remained sterile. It therefore appears that error due to this factor was negligible.

Identity of moulds isolated

Cladosporium has been reported as the dominant air-borne mould in a large number of culture-plate surveys, not only from New Zealand (Dye & Vernon, 1952), but also from England (Hyde & Williams, 1949; Ainsworth, 1952*b*), and various parts of North America (Durham, 1938; Blackaller, 1950). *Penicillium* is also frequently reported as forming an appreciable proportion of the mould colonies grown from air samples. Dye & Vernon found it forming a rather small proportion of their isolates from New Zealand as a whole, but Ambler & Vernon (1951) found it occurring as frequently as *Cladosporium* in a preliminary survey made in the city and suburban districts of Auckland. As many of Dye & Vernon's sampling stations were in small towns, *Penicillium* may possibly be regarded as typical of the air of built-up areas.

Spore showers

Dye & Vernon (1952) reported the periodic occurrence of showers of spores of the dominant genera during all but the winter months. Their criterion of a shower was the presence of over fifty colonies of a given mould per 100 mm. plate. This measure was not applicable to the present work, but an examination of Table 1 shows a high level of *Penicillium* colonies on 17 June 1953. There was a shower of the yeast, *Rhodotorula mucilaginosa*, early in October 1953. High counts of *Cladosporium* were recorded from both sites, particularly the outdoor one, in February and March 1954, but it seems likely from the sustained level that they represent the normal summer spore load rather than intermittent showers. These sudden fluctuations in spore load, particularly during the winter months when the level of aerial antigens is usually low, are of considerable importance to the allergic subject.

II. YEASTS

Although many of the yeasts described in the comprehensive monograph of Lodder & van Rij (1952) are reported as having been originally isolated from air, there is a disappointing lack of surveys of air-borne yeasts. Most workers on the topic of aerial fungus spores ignore yeasts, and those who include them in their results fail to identify them. Previous work in this laboratory has suggested that the non-lipophilic yeast flora of the human skin is derived directly from the air, and is probably a contamination rather than a true resident flora (di Menna, 1954*a*). To investigate this matter further, as well as to obtain information which may be of use in the treatment of the allergic patient, a detailed study of air-borne yeasts has been made.

Samples were taken by the same method as that described in Section I, but at the indoor site only, and between the hours of 10.30 a.m. and 12 noon, instead of at 1 p.m. The series was begun a month earlier than the mould survey, in February 1953, and was run in parallel with it. Ten 100 mm. plates of Sabouraud agar (pH 4) were each exposed to 5 cu.ft. of air on each sampling date. Cultures were incubated at room temperature for 4–5 days. Mould colonies which developed were ignored; the yeast colonies were subcultured into dextrose yeast extract broth (pH 4). Identifications were made by the criteria of Lodder & van Rij (1952). The yeast-like phase of *Cladosporium*, sometimes called 'Torula nigra' is included with the yeasts. The following are the characters upon which its identification was based:

Cultures on solid media: at first cream and mucoid, later becoming pale pink or yellow, and finally blackening. A wide fringe of true mycelium.

Cells oval, sometimes with a ragged mucoid capsule. Many fat globules, some extracellular. Pigmented two-celled spores are formed.

Fermentation: absent.

Sugar assimilation:				
	Glucose	+	Lactose	–
	Sucrose	+	Galactose	–
	Maltose	+		

Assimilation of potassium nitrate: positive.

Three strains which did not fit into any of the defined categories were left unidentified. Some of these may have been new species, but it is more likely that they were aberrant members of unfamiliar groups.

Although relatively little trouble was experienced in the identification

Table 3. *Yeasts isolated from 950 cu.ft. of air at Dunedin* (indoors)

Species	*D. kloeckeri*	*D. subglobosus*	*D. marama*	*S. salmonicolor*	*S. roseus*	*S. odorus*	*C. laurentii*	*C. albidus*	*C. luteolus*	*C. diffluens*	*T. dattila*	*T. candida*	*T. famata*	*C. zeylanoides*	*C. melinii*	*C. scottii*	*T. cutaneum*	*Trichosporon* sp.	*R. glutinis*	*R. mucilaginosa*	*R. minuta*	*R. pallida*	Yeast-like phase of *Cladosporium*	Unidentified	Overgrown	Total
Sampling date																										
25. ii. 53	.	.	.	.	1	.	6	.	.	4	.	.	.	.	.	.	.	.	.	.	.	.	7	.	.	18
11. iii. 53	1	.	.	.	.	.	1	.	.	4	1	.	.	.	.	.	.	.	1	.	.	.	11	.	.	19
25. iii. 53	3	.	.	.	.	.	4	.	.	7	.	.	.	.	1	.	.	.	.	2	.	.	7	.	.	24
7. iv. 53	1	.	.	5	.	.	1	.	.	3	.	.	.	.	.	.	.	.	.	.	.	.	1	.	.	11
22. iv. 53	.	.	.	1	.	.	.	.	.	5	.	.	.	.	.	.	.	.	.	.	.	1	2	.	.	9
20. v. 53	4	.	.	.	.	.	.	.	.	3	.	.	.	1	.	.	.	.	.	.	.	.	3	.	.	11
17. vi. 53	2	1	.	.	.	.	1	.	.	13	.	.	1	.	.	.	.	.	.	.	1	.	.	.	1	20
1. vii. 53	6	2	1	.	.	.	4	.	.	10	.	.	5	.	.	.	.	.	.	1	1	3	1	.	.	34
15. vii. 53	7	1	.	.	.	.	.	1	1	16	.	.	2	.	1	.	1	.	11	.	.	.	.	.	1	42
12. viii. 53	5	1	1	.	.	.	2	1	.	7	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	19
9. ix. 53	3	.	.	.	.	.	.	1	.	5	.	.	2	.	.	.	1	.	2	1	.	.	1	.	.	16
7. x. 53	10	5	1	.	.	.	1	3	.	20	.	1	1	.	.	.	.	.	1	32	4	.	1	.	2	82
28. x. 53	3	3	.	.	.	.	.	1	.	2	.	1	.	.	.	.	.	.	.	.	.	.	1	.	1	12
25. xi. 53	2	.	.	.	1	.	1	2	.	8	.	.	.	.	.	.	.	.	.	3	.	.	.	1	.	18
16. xii. 53	1	.	.	.	.	2	3	.	.	4	.	.	.	.	.	1	.	.	1	.	.	.	1	.	1	14
13. i. 54	1	.	.	.	1	1	5	1	.	11	.	1	.	.	.	.	.	.	1	.	.	.	1	1	.	24
10. ii. 54	6	1	.	.	.	.	8	1	.	1	.	2	.	.	.	.	.	1	2	.	.	.	1	.	.	23
10. iii. 54	9	.	.	.	.	.	.	1	.	3	.	.	1	.	.	.	.	.	.	.	.	.	2	1	.	17
7. iv. 54	6	5	.	.	.	.	3	.	.	2	.	1	.	.	.	.	.	.	.	.	.	.	1	.	.	18
	70	19	3	6	3	3	40	12	1	128	1	7	13	1	2	1	2	1	19	39	6	4	41	3	6	431

of isolates, there was some difficulty in separating *Sporobolomyces salmonicolor* from *S. odorus*. According to Lodder & van Rij, these species are similar biochemically but are differentiated by the presence of mycelium in the former and its absence in the latter. The mycelium produced by the strains described here as *S. salmonicolor* was poorly developed and easily overlooked. The strains labelled *S. odorus* were those in which careful search did not reveal mycelium. *S. odorus* is said to have a characteristic aromatic smell, but none of the strains isolated here had a noticeable odour. The identification of these two species is regarded as tentative.

Except for the lack of ascospores, all strains of *Torulopsis famata* isolated exactly resembled *Debaryomyces kloeckeri*, of which they are the imperfect form (Lodder & van Rij, 1952, p. 418). Those strains of *Torulopsis candida* described here appeared to be the imperfect form of *Debaryomyces subglobosus*.

For the sake of clarity, the yeasts isolated in the mould survey described in Section I are tabulated separately from those isolated in the yeast survey (Tables 3 and 4).

Table 4. *Yeasts isolated from 170 cu.ft. of air in the course of the mould survey at Dunedin, described in Section I*

	Indoor site	Outdoor site	Total
Debaryomyces hansenii	1	.	1
D. kloeckeri	4	2	6
D. subglobosus	1	1	2
D. marama	4	.	4
Sporobolomyces salmonicolor	2	1	3
S. pararoseus	1	.	1
Cryptococcus laurentii	2	12	14
C. albidus	2	7	9
C. luteolus	1	.	1
C. diffluens	26	21	47
Torulopsis candida	.	2	2
T. famata	1	1	2
Candida melinii	.	1	1
Rhodotorula glutinis	2	1	3
R. mucilaginosa	2	5	7
R. flava	1	.	1
R. minuta	2	3	5
R. pallida	2	1	3
Trichosporon cutaneum	1	.	1
Yeast-like phase of *Cladosporium*	7	4	11
Unidentified or overgrown	3	2	5
	65	64	129

RESULTS

Four hundred and thirty-one yeasts were isolated from 950 cu.ft. of air by means of nineteen regular and equal samplings between February 1953 and April 1954.

Over 96 % of the yeasts isolated belonged to four groups. Forty-two per cent were non-pathogenic *Cryptococcus* species (181 isolates); 26·2 % were *Debaryomyces* (113 isolates, including the imperfect forms, *Torulopsis candida* and *T. famata*); 18·6 %, the *Sporobolomyces–Rhodotorula* group; and

9·5 % the yeast-like phase of *Cladosporium*. Four strains of species of *Candida* were isolated, three of *Trichosporon* species, and one of *Torulopsis dattila*. Of the remaining nine strains, three were unidentifiable, and six were too overgrown by moulds to be recovered.

Forty-six per cent of the yeasts belonged to two species, *Cryptococcus diffluens* (128 isolates) and *Debaryomyces kloeckeri* (70 isolates). Both these species occurred regularly; their preponderance was not due to a few showers. *Cryptococcus diffluens* was isolated each day samples were taken, and *Debaryomyces kloeckeri* on all but two of the collecting days.

None of the yeasts showed marked seasonal distribution. A total of twelve strains of *Sporobolomyces* were isolated, none of them during the May–October period. Although this is suggestive of a summer incidence or fruiting period for this genus, the numbers are too few for a conclusion to be drawn.

Detailed results are given in Table 3. The 129 yeasts isolated in the course of the mould survey described in Section I of this paper, which are essentially similar in species and proportions of species to those isolated in the main yeast survey, are listed in Table 4.

In the yeast survey, where 5 cu.ft. of air were sampled per plate, the concentration of viable yeasts in the air averaged one cell or clump of cells to 2 cu.ft. On the other hand, in the mould survey where 1 cu.ft. of air was sampled per plate, 129 yeasts were isolated from 170 cu.ft. of air, giving a concentration of the order of one cell per cu.ft. There are two explanations for this disparity, not necessarily exclusive of each other. One is that many yeast colonies were overgrown by moulds on the more heavily inoculated plates; the other that the samples for the yeast survey were taken at 11 a.m. and those for the mould survey at 1 p.m., and that a rise in count occurred between these hours.

Discussion

Comparison of the present findings with those from a similar but smaller survey of air-borne yeasts made in Dunedin in the period July–September of 1952 (di Menna, 1954*a*) shows only minor differences in the proportions of genera encountered and the species isolated. There are, however, some discrepancies between these findings and those of Connell & Skinner (1953) in Washington. These workers found 'black yeasts' dominant (44·3 %), and the *Sporobolomyces–Rhodotorula* group next in importance on their air-inoculated plates. Non-pigmented fermenters and non-fermenters, which occurred in approximately equal proportions, comprised only 22·5 % of their total isolates. In the survey reported here *Cryptococcus* and *Debaryomyces* species (which Connell & Skinner would have placed in their unpigmented, non-fermenting group) made up over two-thirds of the strains recovered. Fermenting yeasts were represented by one isolate of *Torulopsis dattila*. Connell & Skinner identified only one of their groups of yeasts as far as species rank, so that it is not possible to make detailed comparisons, but it appears that there may be marked regional differences in the species of yeasts in the air.

Allergy

Harsh & Allen (1945) reported that 4% of allergic patients in San Diego reacted to extract of 'Torula', and 6% to an extract of 'yeasts'. Whilst the proportion of air-borne yeasts to moulds is usually low, there are occasions, such as in still, snowy weather, or during 'showers', when they form a large part of the spore load and may cause a reaction in sensitive patients.

Contamination

When yeasts are isolated from material which has been subjected to actual or potential aerial contamination, it is usually difficult to assess their significance. From the results obtained in this survey it appears that, in this district at least, *Debaryomyces* sp., *Rhodotorula* sp., the yeast-like phase of *Cladosporium*, and some species of *Cryptococcus*, particularly *C. diffluens*, must be suspected of being aerial contaminants. As these were the groups of yeasts isolated from human skin in a survey made in Dunedin (di Menna, 1954*a*) it must be concluded that the non-lipophilic yeasts isolated from skin do not constitute a true flora but are contaminants from the air.

Ecology

Obviously there is some situation in which the air-borne yeast is not a contaminant. Three hypotheses may be advanced as to the origin of these organisms. One is that they are the most frequently occurring yeasts of the area, another that they are a group particularly resistant to desiccation and ultra-violet radiation, and a third that they are the yeasts of one particular environment.

The first hypothesis may probably be dismissed. If aerial yeasts are an undiscriminated selection, it might be expected that they would include soil yeasts, *Saccharomyces cerevisiae* in towns where bakeries and breweries handle large amounts of commercial yeast, and, in thickly populated areas, *Candida albicans*. A short survey of the yeasts of Dunedin soils, made in this laboratory, has shown the predominant yeasts to be *Cryptococcus albidus*, which is relatively infrequent in air, and *C. terreus* (di Menna, 1954*b*), which is absent. Neither *Saccharomyces cerevisiae* nor *Candida albicans* was found in the air samples. A special survey was made of brewery air to find the degree of contamination by *Saccharomyces cerevisiae*. Air was sampled by means of the slit sampler in the brewing squares, the gyles and the yeast storage room. Of the eighty-four yeasts isolated only one was *S. cerevisiae*, and it occurred on a plate which had been manipulated by hands contaminated with inoculated wort. The remaining isolates belonged to the usual air-borne genera.

The second hypothesis appears more tenable. A series of experiments was carried out to investigate the resistance of air-borne yeasts to desiccation and ultra-violet irradiation. Suspensions of yeasts were dropped on sterile filter-paper disks which were then dried at room temperature and stored in the dark. *Debaryomyces kloeckeri*, *Sporobolomyces salmonicolor*, *Cryptococcus diffluens* and the yeast-like phase of *Cladosporium* were used as examples of air-borne yeasts, with *Saccharomyces cerevisiae*, *Torulopsis colliculosa*, *Candida albicans* and *C. krusei* as controls. *Saccharomyces cerevisiae* failed to survive the drying test—a disk dropped into broth the day after drying yielded a sterile culture. The last positive culture from *Candida albicans* appeared two weeks after drying, from *Torulopsis colliculosa* 5 weeks, and from *Candida krusei* 10 weeks. *Debaryomyces kloeckeri* survived 10 weeks, and *Sporobolomyces salmonicolor*, *Cryptococcus diffluens* and the yeast-like phase of *Cladosporium* were still all viable after 15 weeks when the experiment was concluded. The same group of yeasts was subjected to irradiation with ultra-violet light from a hot-source Hanovia lamp. They showed essentially the same degree of tolerance to ultra-violet light as to desiccation. This suggests that the ability to withstand the effects of drying and sunlight is an important factor in determining whether or not a yeast is distributed aerially. Unfortunately the method used in this survey gives no indication of the numbers of dead yeast cells in the air, and these, to the allergic subject, are as important as viable organisms.

Insufficient evidence is as yet available either to support or disprove the third hypothesis, that air-borne yeasts may be those belonging to a particular environment. Some minor pieces of information have suggested that the air-borne yeast groups are the same as those which may be isolated from vegetation, but the topic requires further investigation before any conclusions can be reached.

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EXPERIMENTAL STUDIES ON THE ECOLOGY OF FUNGI IN SOIL

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(With Plate 4 and 3 Text-figures)

When fungal conidia and sporangiospores are placed on soil they decompose, this being true even of the spores of organisms that are native to the soil. Natural plant materials are more effective than soluble chemical amendments in reducing the rate of this decomposition. Partially decomposed plant materials as found in soil are also effective. Such plant materials can be utilized in the soil by both native and alien fungi. Only native fungi, however, were able to make the fullest use of them in this situation, by successful colonization and survival for longer than 6 months. This was due to a high degree of competitive saprophytic ability under the conditions found in soil. The bearing of these findings on the ecology of fungi in soil is discussed.

INTRODUCTION

The difficulty of successfully introducing alien organisms into soil has been repeatedly demonstrated (Sanford, 1933; Katznelson, 1940; Anwar, 1949), the introduced organism dying out rapidly. This fact emphasizes the ecological distinction between 'soil-inhabitants' and 'soil-invaders' drawn by Waksman (1917). Butler (1953*a*), on the basis of comparative studies of soil-borne pathogens, supported Garrett's (1950) suggestion that the properties underlying this distinction are to be found in the competitive saprophytic powers of the organisms. In the work to be described here the saprophytic ability of organisms native to a selected soil was compared with that of alien organisms.

MATERIALS AND METHODS

The soil selected for study was a light garden loam, pH 6·4 (loss in weight on ignition: 6·28 %). The samples were taken from the top 2 in. of the plot, which was kept free of macroscopic vegetation but otherwise untreated for 9 months before the investigation began and throughout the course of the experiments.

Twelve fungi, six native and six alien were used. Of these *Mucor silvaticus* Hagem, *Fusarium roseum* sensu Snyder & Hansen, *Cladosporium cladosporioides* de Vries, *Penicillium roqueforti* Thom, *Trichoderma viride* Fr. and *Monotospora daleae* Mason are described as natives, being isolated from the soil used, by the soil plate method of Warcup (1950). Of the twenty-six isolates made by this method, these six were chosen for study, without reference to the possession of any special characters. The six aliens, *Rhizopus sexualis* (Smith) Callen, *Aspergillus niger* v. Tiegh., *Botrytis cinerea* Fr., *Penicillium digitatum* Sacc., *Trichothecium roseum* Link, and *Stemphylium* *sarcinaeforme* (Cav.) Wilts., were not isolated from the soil used, and had been subcultured on artificial agar medium for upwards of 12 months. During the course of the experiments the twelve fungi were maintained in tubes of autoclaved soil and periodically examined for changes in growth rate, pigmentation, sporulation and morphological features, on a number of different media. The organisms retained their original characters.

Except where air-dry soil was required, samples of soil were obtained not more than 4 hr. before use and passed through a 3 mm. mesh sieve to remove gravel and stones. Soil-dishes were prepared by packing the sieved soil firmly as a 4–5 mm. layer in the base of a Petri dish. A clean microscope slide was lightly pressed on to the soil to leave a slight depression; this *slide-area* was used for subsequent observation by the contact-slide method. The lid was then replaced on the dish. Where sterile soil was required the soil-dish, wrapped in heavy paper, was autoclaved at 121° C. for 30 min. Inoculation of the soil was carried out by uniformly spreading 1 ml. of a spore suspension of the test-fungus over the slide-area with a fine pipette. The dishes were incubated on the laboratory bench at 15·8–21·3° C. No attempt was made to maintain the soil at a constant moisture level. The moisture level varied between 43 and 60% water holding capacity (W.H.C.) at the beginning and between 36 and 49% W.H.C. at the end of the experiments.

The inoculated soil-dishes were examined by the modification of the contact-slide method (Rossi, 1928) adopted by Hessayon (1953). A clean slide was pressed evenly on the slide-area for 20 sec., removed and allowed to become air-dry. After being slowly passed 4 times through a bunsen flame the slide was stained for 60 sec. in phenolic rose bengal (Conn, 1932) at 95° C., rinsed in cold water and examined as a water mount. Duplicate slides were taken except where it is stated otherwise.

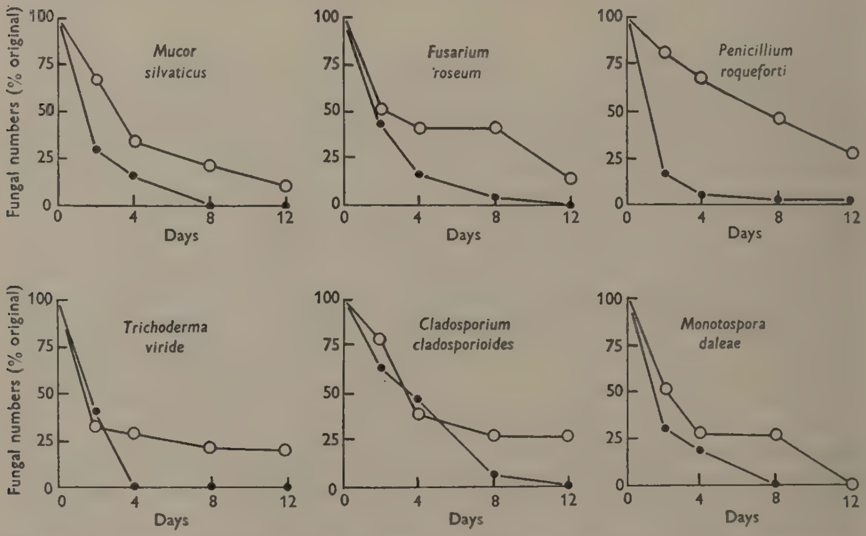
In the quantitative contact-slide method (Hessayon, 1953) a square 2 × 2 cm. was scratched on the surface of the slide. Spore counts within this microquadrat were made using a mechanical stage. Slides were prepared from triplicate soil-dishes when this method was used. Contact-slides have previously been used quantitatively by Verplanke (1932), Garrett (1938) and Timonin (1940). Since Demeter & Mossel (1933), discussing this procedure, consider this method only approximately accurate, the plating method was also used here as a parallel measure. The plates were prepared by the standard method from 30 g. inoculated soil at a dilution of 1 in 5000. For each treatment 10 g. of soil were taken from the inoculated soil of each of three dishes to give the soil sample. The medium recommended by Smith & Dawson (1944), containing one part rose bengal in 15,000, was used, four plates being poured for each soil sample. In each experiment observations of uninoculated, but otherwise comparable, control soil-dishes were made. The results of these observations are given only when they affect the interpretation of the inoculated experimental treatments.

Plant material of two kinds was added to soil-dishes: leaves from vegetative shoots of *Dactylis glomerata* L., and internodal regions of stolons of *Trifolium repens* L. These were stored and weighed in an air-dry condition, and moistened for use by placing in a humid atmosphere for 24 hr. When required sterile the material was exposed to propylene oxide vapour for 30 hr. (Hansen & Snyder, 1947). To determine whether the test-fungus had colonized the plant pieces these were surface-sterilized in a 7 % solution of calcium hypochlorite for 20 min. (Mead, 1933), washed, and plated on agar. The rose bengal agar used prevented overgrowths and enabled the slow-growing species, if present, to be recognized.

RELATION OF THE NATIVE SPECIES TO THE SOIL

Experiment 1

Dishes of fresh, sieved soil were inoculated separately with spores of the six native fungi, and observations made over 12 days by the two methods described. Autoclaved soil controls were used. On autoclaved soil the spores of all fungi germinated and made extensive growth. With the exception of *Monotospora*, all species sporulated normally, and increased numbers were recorded by both methods. *Monotospora* remained vegetative and only the plate method showed an increase in numbers. Chlamydospore formation occurred in the mature hyphae of *Mucor*.



Text-fig. 1. Reduction in the numbers of spores of native fungi added to soil. ○—○, observations by the plate method; ●—●, observations by the contact-slide method.

The results of the observations in unautoclaved soil are shown in Text-fig. 1. No germinated spores were seen. The decrease in numbers is due to the digestion of the dormant spores as they lie in the soil. Spores of both *Mucor* and *Trichoderma* were seen devoid of contents, remaining recognizable as empty spore walls. The observation of successive stages in the disappearance of the multicellular *Fusarium* conidia (Pl. 4, fig. 1*b*) gives further evidence of this digestion. A more intense development of

Actinomycetes and bacteria was noted in the inoculated soil as compared with the uninoculated controls. Since survival as observed by the dilution method was better than that shown by the contact-slide method, it is possible that there are habitats below the soil surface in which spore decomposition is less pronounced.

Experiment 2

Soils amended to a level of 3% by weight with dextrose, sodium nitrate, and the two plant materials, respectively, were used to determine the effect of these substances on spore digestion in the soil. The pure chemicals were added as sterile solutions to air-dry soil, bringing the soil to 45% W.H.C. The plant materials were finely divided, moistened, sterilized and mixed with the air-dry soil which was subsequently adjusted to the moisture level of the other treatments. Soil-dishes were prepared from these soils and separately inoculated with the spores of the six fungi. Unamended soil was used in a control treatment. Observations were made over 8 days, Text-fig. 2 giving the results of spore counts on the contact-slides. The plate method gave comparable results confirming the trends indicated by the direct method.

In unamended soil, digestion of the spores took place as in Exp. 1. The presence of nitrate reduced slightly the rate of decomposition, but no germination was seen. In the soil amended with dextrose, germination of both *Fusarium* and *Mucor* occurred. The germlings of *Fusarium* had, however, disappeared completely after four days, whereas those of *Mucor* continued to grow and soon sporulated. The growth was of an ephemeral nature, and decomposition of the sporangiophores, chlamydospores, and hyphae followed quickly (Pl. 4, fig. 2) so that the spore numbers decreased after the fourth day. The rate of disappearance of the other four fungi was increased slightly by the presence of dextrose.

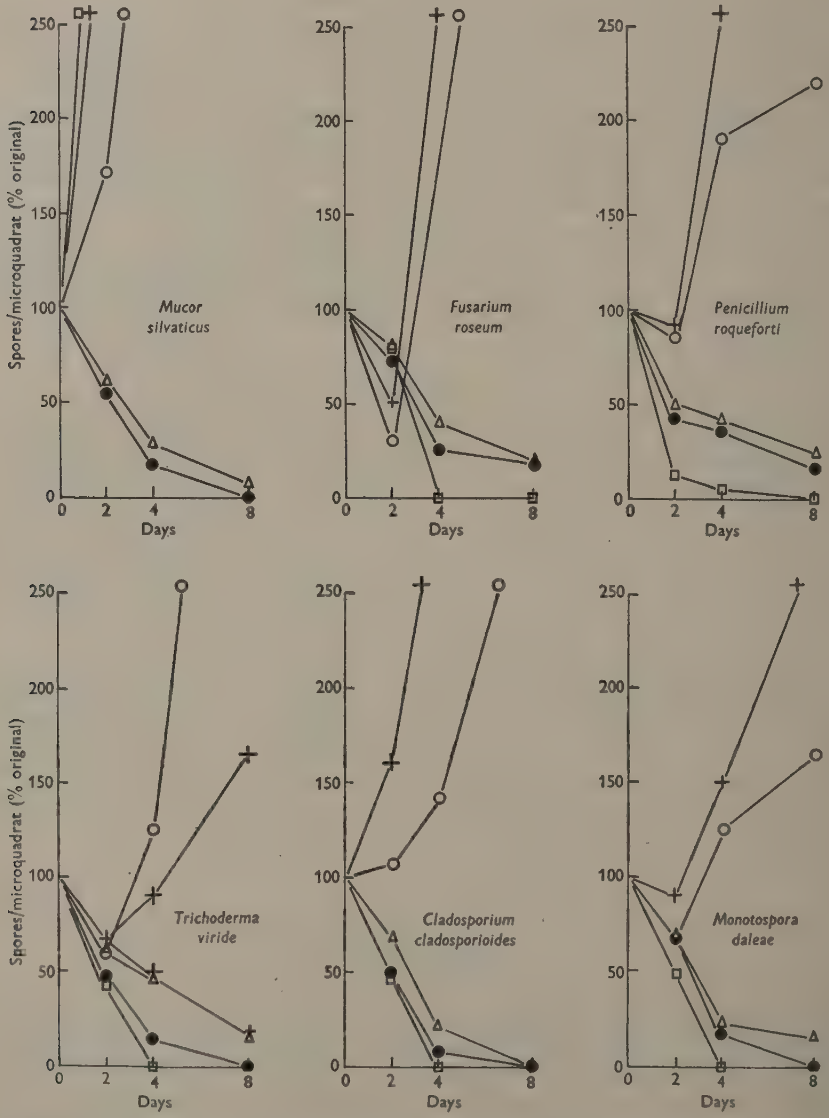
Germination, growth and sporulation occurred in all six fungi in soil amended with plant material. Sporulation was, in general, of the normal type. The early stages of fertile *Trichoderma* hyphae frequently had a Gliomastix-like appearance, the same hyphae bearing normal conidiophores more distally (Pl. 4, fig. 3). Despite the good growth, decomposition and loss of contents was observed in the newly produced spores of *Mucor*, *Fusarium*, *Penicillium roqueforti* and *Trichoderma*.

COMPARISON OF NATIVE SPECIES WITH ALIENS

Experiment 3

Method (*a*). Soil-dishes were prepared, and sterile 10 mm. pieces of plant material pressed flat to the soil surface on the slide-area at least 20 mm. apart. The soil surface was inoculated, separate sets of dishes being used for each fungus. The contact-slide method, used for observations over 8 days, was modified by marking on the reverse of the slide with a writing diamond those areas of the slide coming into contact with pieces of plant material so that they could be recognized during the examination of the slide.

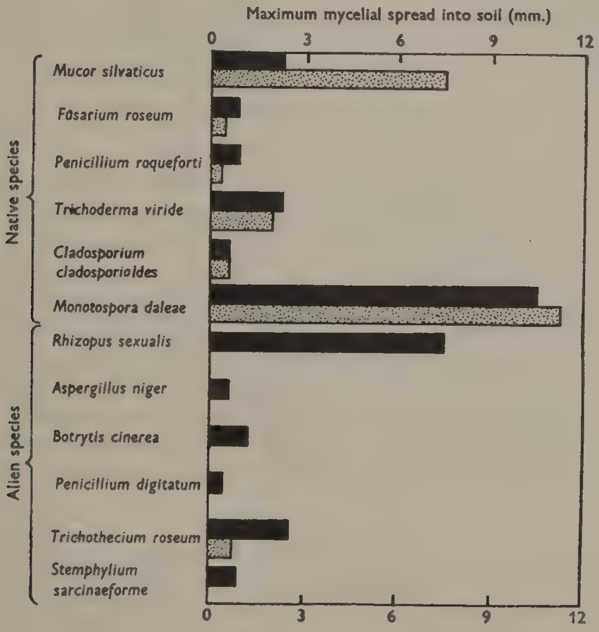
Spores of the native species lying on the surface of the plant material germinated to give fertile colonies. Sporulation was sparse in *Penicillium roqueforti* and *Monotospora*, moderate in *Trichoderma*, and intense in *Mucor*, *Fusarium* and *Cladosporium*. Spores which came to rest on the soil surface or on pieces of clean cover-glass placed on the soil failed to germinate and



Text-fig. 2. Effect of the presence of amendments on the numbers of spores of native fungi added to soil. +, clover internode; ○, grass leaf; △, sodium nitrate; □, dextrose; ●, unamended soil.

eventually disappeared. Spore digestion and hyphal breakdown were common even on the surface of the plant materials.

Spores of the alien species also disappeared from the soil and the pieces of glass. Germination was not observed in *Aspergillus* on clover, or in *Botrytis* and *Penicillium digitatum* on grass, the spores in these treatments disappearing. In the other alien species, the spores on the surface of the plant materials germinated, but developed poorly compared with the native species, sporulation being sparse and local. Resident soil species were more dominant in the materials here.



Text-fig. 3. Maximum mycelial spread into soil from a natural food-base. ■, food-base previously colonized by the fungus; ▦, fungus added to the food-base in soil as a spore suspension.

Measurements of the maximum distance of recognizable mycelial spread from the food-base into the soil appear in Text-fig. 3. On the eighth day after inoculation six plant pieces from each treatment were surface-sterilized and plated to determine which of the test species had colonized. The results are given in Table 1.

Method (*b*). The technique was similar to that in method (*a*); the sterilized materials, however, were inoculated in sterile Petri dishes and incubated for 5 days at laboratory temperature before being added to the soil surface, i.e. the added plant material was already colonized.

The native species showed a more intense development here than in the previous method, and other soil species were less frequently encountered on the plant pieces. Otherwise there was no noticeable difference in the results from the two methods. Spore digestion still occurred, and only in *Mucor* was the extent of spread into the soil very different from that obtained previously. In this method its spread was considerably less. It is probable that the organism, being ephemeral, had passed its optimum stage of development in the inoculating period.

Table 1. *Colonization of plant pieces lying on the surface of soil*

Natives	Grass	Clover	Aliens	Grass	Clover
Mucor silvaticus	6	6	*Rhizopus sexualis*	0	0
Fusarium roseum	4	5	*Aspergillus niger*	0	0
Cladosporium cladosporioides	3	6	*Botrytis cinerea*	0	0
Penicillium roqueforti	4	3	*Penicillium digitatum*	0	0
Trichoderma viride	5	4	*Trichothecium roseum*	0	0
Monotospora daleae	4	4	*Stemphylium sarcinaeforme*	1	0

Note—the numbers are out of a maximum of six.

The alien fungi, in contrast, presented results quite different from those seen in uncolonized materials. Growth and sporulation in all species was more vigorous, and spread of some of these fungi into the soil was relatively extensive (Text-fig. 3).

The results of this experiment demonstrate that both native and alien fungi possess the ability to produce spores on plant material lying in soil, and to spread from this food-base into the soil. The main distinction between these two categories of organism lies in the ability to colonize successfully and to make the maximum use of decomposable materials lying in the soil habitat; aliens are relatively unsuccessful at obtaining an initial hold in the food-base in competition with resident soil organisms.

Experiment 4

The ability of natives and aliens to colonize plant materials beneath the surface of soil was tested. The fresh, sieved soil was packed firmly to a depth of $2\frac{1}{2}$ in. in 3 in. plant pots. Three pieces of plant material were placed on the surface of the soil, inoculated with a spore suspension of the fungus, and immediately covered with about 1 in. of soil which was then firmly packed down. After 8 days' incubation at laboratory temperature the soil was carefully excavated, and the pieces removed, surface-sterilized and plated. Six pieces of material were used for each fungus. The number of pieces giving rise to colonies of the inoculated fungus are shown in Table 2; the aliens were completely unsuccessful, while the natives effectively colonized a large number of plant pieces, being only slightly less successful than when the plant pieces lay on the soil surface.

Experiment 5

Since partially decomposed plant material is more common in soil than the fresh material, the ability of the fungi to utilize this substrate was examined. The partly rotted, but still recognizable, pieces were taken from the soil, and cut into 10 mm. lengths. One lot, series N, was used in the natural state, while a second, series S, was sterilized before use.

Method (*a*). Pieces from both series were added to the surface of prepared soil-dishes and inoculated. Observations, as in Exp. 3, were made over the course of 8 days. There was no obvious difference between the development on the two series of pieces. With the exception of *Rhizopus* the spores of all twelve fungi germinated and produced further spores on the added material. The intensity of sporulation in natives was not consistently heavier than that in aliens, *Trichoderma* producing but few conidia, whereas *Penicillium digitatum* and *Trichothecium* both produced abundant spores. On the eighth day six pieces of material from each series were taken for each fungus and tested for colonization by the inoculated species. The results are presented in Table 3.

Table 2. *Colonization of plant pieces below the soil surface*

Natives	Grass	Clover	Aliens	Grass	Clover
Mucor silvaticus	4	5	*Rhizopus sexualis*	0	0
Fusarium roseum	3	4	*Aspergillus niger*	0	0
Cladosporium cladosporioides	4	4	*Botrytis cinerea*	0	0
Penicillium roqueforti	3	4	*Penicillium digitatum*	0	0
Trichoderma viride	4	2	*Trichothecium roseum*	0	0
Monotospora daleae	3	5	*Stemphylium sarcinaeforme*	0	0

Note—the numbers are out of a maximum of six.

Table 3. *Colonization of partially decomposed plant pieces*

	Soil		Dish and sterile water	
	Sterilized	Untreated	Sterilized	Untreated
Natives				
Mucor silvaticus	3	1	6	4
Fusarium roseum	6	4	6	6
Cladosporium cladosporioides	0	0	6	0
Penicillium roqueforti	6	4	6	5
Trichoderma viride	5	4	6	6
Monotospora daleae	5	3	6	3
Aliens				
Rhizopus sexualis	0	0	0	0
Aspergillus niger	0	0	6	0
Botrytis cinerea	0	0	4	0
Penicillium digitatum	0	0	5	0
Trichothecium roseum	0	0	6	0
Stemphylium sarcinaeforme	2	1	6	2

Note—the numbers are out of a maximum of six.

Method (*b*). For each test-fungus, six plant pieces from each series were prepared, placed in a humid atmosphere in sterile Petri dishes, and inoculated with a spore suspension of the fungus. At the end of 8 days the pieces were tested for colonization as in the previous method. Table 3 shows the numbers giving colonies of the appropriate fungus.

Again the prominent distinction between the two categories of fungi was in their ability to colonize the substrate deeply in the presence of competitors. The intensity of surface activity was not associated with this property. The colonization of the series S pieces in Petri dishes by all the fungi but *Rhizopus* implies that the substrate itself was not unsuitable for these other organisms.

Experiment 6

The previous experiments support the view that saprophytic ability is important in the ecology of native soil fungi. It was desired in this experiment to determine whether the distinction between the two types of organism was due to differences in absolute saprophytic ability or in competitive saprophytic ability.

Uniform pieces of clover internode were prepared and sterilized, and, after evaporation of the propylene oxide vapour, placed in twelve sterile Petri dishes, to each of which was added 20 ml. of a spore suspension of the appropriate test-fungus. After standing in contact with the suspension for 7 min. the pieces were taken out and placed on moist filter-paper in autoclaved Petri dishes, twelve pieces in each Petri dish, so that a distance of at least 5 mm. separated the individual pieces. The dishes were incubated at 20° C.

Observations were made 24, 48, 72 and 96 hr. after inoculation by surface-sterilizing, for each fungus, six pieces at 5, 10, 20 and 30 min., respectively. After plating and incubating, the number of these pieces giving colonies of the inoculated fungus were counted. After 96 hr. colonization all twelve species completely withstood the 30 min. sterilization. The results for the observations over the first 3 days are given in Table 4.

Table 4. *Rate of colonization of plant pieces under sterile conditions*

Days of colonization ...	1				2				3			
Minutes of sterilization	5	10	20	30	5	10	20	30	5	10	20	30
Natives												
Mucor silvaticus	6	6	6	5	6	6	6	6	6	6	6	6
Fusarium roseum	6	4	4	2	6	6	4	5	6	6	6	6
Cladosporium cladosporioides	2	3	2	0	6	6	6	3	6	6	6	6
Penicillium roqueforti	0	0	0	0	6	6	5	5	6	6	6	6
Trichoderma viride	0	2	0	0	6	6	6	5	6	6	6	6
Monotospora daleae	0	0	0	0	6	6	5	5	6	6	6	6
Aliens												
Rhizopus sexualis	1	1	0	0	6	6	5	4	6	6	6	6
Aspergillus niger	1	1	0	0	5	6	2	1	6	6	6	6
Botrytis cinerea	1	0	0	0	6	6	6	5	6	6	6	6
Penicillium digitatum	2	0	0	0	6	6	4	4	6	6	6	6
Trichothecium roseum	0	0	0	0	2	3	2	0	6	5	4	4
Stemphylium sarcinaeforme	3	2	0	0	6	6	6	6	6	6	6	6

Note—the numbers are out of a maximum of six.

Although the most rapid colonizers were natives and the weakest were aliens, this differentiation was not complete, as may be seen by totalling the number of pieces colonized by each fungus during the experiment. Half of the aliens in fact colonized more rapidly than either *Penicillium roqueforti* or *Monotospora*. Thus the rate at which an organism colonized the material under the conditions provided here bore no direct relation to its ability to colonize that material in soil. This supports the theory that competitive saprophytism is the important factor in soil relations.

Experiment 7

Sterile pieces of grass leaf and clover internode were separately inoculated with the test-fungi, incubated for 5 days, and then buried in prepared soil to a depth of 1 in. The plant pots containing the soil were incubated at a temperature varying from 15·8 to 23·4° C. and watered at intervals. Observations were made over the course of 36 weeks by taking up six pieces of each type of material for each fungus, washing in sterile water, and plating to determine the number giving colonies of the inoculated fungus. The counts are given in Table 5.

Table 5. *Survival in previously colonized plant pieces buried in soil*

	Clover								Grass				
Time of observation in weeks	2	4	8	12	18	24	30	36	2	4	8	12	18
Natives													
Mucor silvaticus	6	6	6	6	6	6	6	6	6	6	6	6	—
Fusarium roseum	6	6	6	6	6	6	6	6	6	6	6	—	—
Cladosporium cladosporioides	6	6	6	6	6	6	6	6	6	6	6	—	—
Penicillium roqueforti	6	6	6	6	6	6	6	6	6	6	6	—	—
Trichoderma viride	6	6	6	6	6	6	6	6	6	6	6	6	6
Monotospora daleae	6	6	6	6	6	6	6	6	6	6	6	6	—
Aliens													
Rhizopus sexualis	0	0	0	0	0	0	0	0	4	0	0	—	—
Aspergillus niger	6	6	2	0	0	0	0	0	6	5	—	—	—
Botrytis cinerea	6	3	0	0	0	0	0	0	6	0	—	—	—
Penicillium digitatum	0	0	0	0	0	0	0	0	0	0	0	—	—
Trichothecium roseum	6	5	2	0	0	0	0	0	6	6	0	0	—
Stemphylium sarcinaeforme	6	6	6	4	0	0	0	0	6	6	6	6	—

Note—the numbers are out of a maximum of six. A dash indicates that decomposition had proceeded so far as to make handling of the material impossible.

The most definite results were given by clover, in which all the natives showed complete survival to the end of 36 weeks, whereas after 18 weeks no alien colony could be obtained although the material remained largely undecomposed. Decomposition of grass was more rapid and observations could not be continued for longer than 18 weeks. However, the results obtained indicate that the natives survive for at least as long as the material remains coherent, while the aliens, with one exception, showed reduced survival rates before decomposition was complete.

In a parallel experiment, instead of adding colonized plant material to the soil, sterile pieces of grass and clover were added, inoculated with a spore suspension of the fungus, and immediately covered with a 1 in. layer of soil. The results confirmed those already obtained, in that the alien organisms completely failed to colonize the material, whereas each native organism showed survival in at least three of the six pieces of clover after 36 weeks, and in the grass so long as it was recognizable.

DISCUSSION

Waksman (1927) had pointed out that a decrease in the numbers of fungi in soil occurred following the addition of dextrose. Neilson-Jones (1941) has shown that addition of compost to soil overcomes the toxicity better than pure chemical amendments. Nelson (1944) and Skinner (1953) also counteracted toxicity towards fungi by adding organic matter. Although Shumakov (1941) has noted that water soluble substances may be absorbed by the soil from decomposing litter, spores near, but not touching, the plant material in these experiments were not affected by its presence. It would appear to be the solid phase that is the important protective agent in this situation.

Two types of utilization of plant material in soil were observed. In one the fungus exists as a fertile mycelium restricted to the surface, and making no effectual penetration of the substrate. In the other the mycelium colonizes deeply enabling the fungus to maintain itself more permanently. Only natives were found to effect deep colonization of materials in contact with the soil. That the lack of success of the aliens in this situation was not due to any inherent inaptitude was shown by pure culture experiments. These results support the view of Garrett (1950) that success in the soil is assignable to competitive saprophytic ability. If helped by previous colonization the aliens in general proved no less effective than the natives in sporulating on the surface of material in soil, and in making mycelial spread through the soil from the food-base. Butler (1953*a*) has pointed out that competitive saprophytic ability and the capacity to spread outwards into the soil are two unconnected properties.

Some fungi can colonize a material without having the ability to survive in it (Downie, 1943), and others, although unable to colonize, yet can survive successfully (Butler, 1953*b*). For this reason the natives and aliens were compared in their ability to survive in colonized material lying in soil. The aliens disappeared from the plant materials in less than 6 months, whereas the natives showed full survival after 9 months where the material had not been completely decomposed. Here, at least, the ability to colonize and to survive are correlated.

A fungus would appear to need three attributes if, in the absence of repeated heavy inoculations, it is to be consistently isolated from a soil: the spores, or other source, must be able to commence activity; the mycelium must make successful vegetative growth; and the fungus must be able to survive any inimical conditions to which it is subjected. A failure in any of these phases will limit the fungus to, at most, a temporary existence in the soil. Sources of fungi, including soil-inhabitants, are present as spores in air (Pady & Kelly, 1953). Whether these become established, after being deposited on the soil, will depend on the availability of suitable decomposable substrates and on the relation between the properties of the fungus and the operative environmental factors. Lacking the ability to colonize the material successfully, the fungus might, by superficial sporulation, reduce the apparent rate of disappearance of its spores, but would eventually suffer from the competition of more highly adapted organisms. It is possible that a soil-inhabitant is not found in a certain soil because of the failure of the organism to fulfil the necessary conditions in that particular environment. Consequently, while the term *soil-inhabitant* is a definition in terms of habitat, the description *native soil fungus* may be useful in a more restricted sense, implying an ecological fitness for the limited environment under discussion.

The diversity of habitats and decomposable materials found in soils is wide, and since efficiency in different situations may be conferred by different mechanisms, it may be arbitrary to attempt to ascribe success in soil generally to specific physical or physiological characters. The suggestions that an autecological approach be adopted (Garrett, 1952) and that the fungi be studied in relation to the crude organic matter at the soil surface (Chesters, 1949) seem well suited to this type of problem.

The writer wishes to thank Prof. C. W. Wardlaw, under whose supervision this work was carried out, and Mr E. Ashby, who prepared the photographs. Mr G. Smith kindly identified *Penicillium roqueforti*.

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EXPLANATION OF PLATE 4

Fig. 1. (*a*) Conidium of *Fusarium roseum* on addition to soil. (*b*) Conidium of *F. roseum*, partially digested, after two days in soil. × 1000.

Fig. 2. Structures of *Mucor silvaticus* after 4 days in soil amended with dextrose. *A*, mycelium lacking cytoplasm; *B*, deeply staining chlamydospore; *C*, decomposing chlamydospore; *D*, sporangiospore lacking contents. × 500.

Fig. 3. (*a*) 'Gliomastix'-like sporulation of *Trichoderma viride* in soil amended with grass leaf material. (*b*) Normal *Trichoderma* conidiophore, more distally on the same hypha. × 500.

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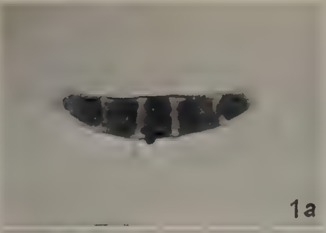
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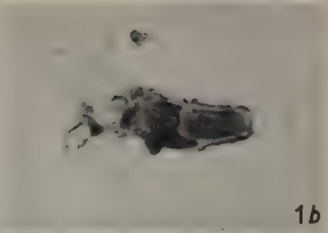
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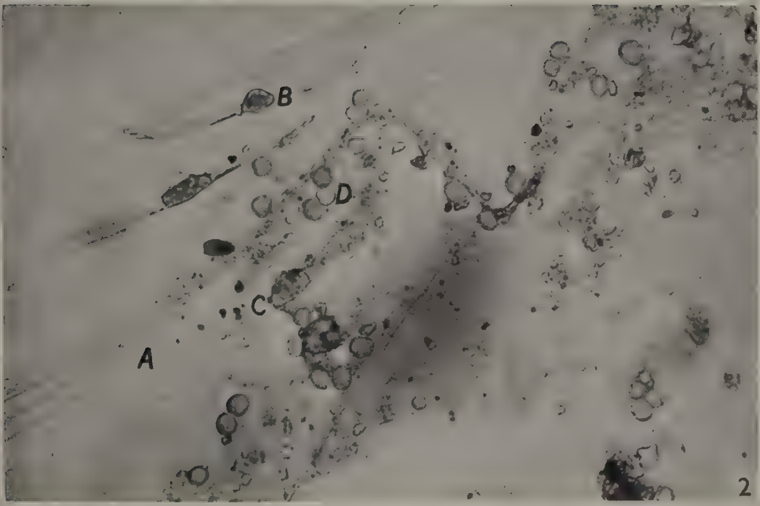

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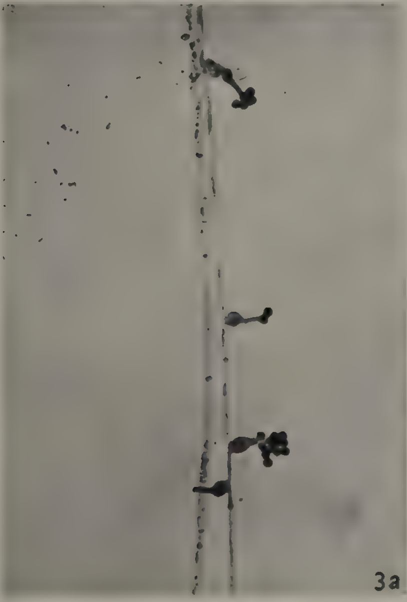
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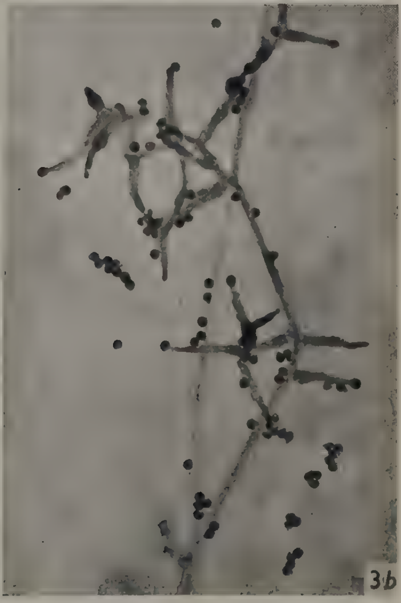
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Trans. Brit. mycol. Soc. **38** (2), 143–156 (1955).

A NEW HYALINE SPECIES OF *VERTICILLIUM: V. INTERTEXTUM* SP.NOV.

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(With 5 Text-figures)

An isolate of *Verticillium*, obtained from a wilted Japanese maple tree in Massachusetts, U.S.A., formed septate, branching mycelium which, when growing subaerially or aerially, became aggregated into mycelial strands or 'ropes' from which the verticillately branched conidiophores developed. When grown on Dox's agar in darkness the prostrate mycelium was hyaline, but in daylight it was distinctly orange-pink.

Maximum growth in culture was at 25° C. and at pH 7·2. Good growth occurred on media containing sucrose, dextrose, maltose or glycerine with a maximum on maltose. Variation of the nitrogen source indicated that growth was best on media containing sodium nitrate.

The fungus, although re-isolated from inoculated specimens of *Acer palmatum*, did not induce wilt in this plant nor in a wide range of other plants usually susceptible to infection by *Verticillium*.

The organism is accorded specific rank and is named *Verticillium intertextum* sp.nov.

Introduction

In 1950 we received from Dr F. L. Howard of the University of Rhode Island, U.S.A., a culture of *Verticillium* which was hyaline since it did not produce such dark-walled resting structures as microsclerotia, carbonized hyphae and chlamydospores. This fungus, which in the present work will be designated VM, had been sent to the University of Rhode Island by Dr Malcolm A. McKenzie, Director of Shade Tree Laboratories, The University of Massachusetts, who stated that he had isolated it from diseased Japanese maple *Acer palmatum* Thunb. from Easthampton, Massachusetts, and had identified it as a species of *Verticillium*. He was kind enough to send us from the same infected tree from which he had made his original isolation freshly cut maple twigs which showed dark green discoloration of the wood typical of the *Verticillium* disease of maple described by Zimm (1918), van der Meer (1925) and Carter (1940). From them a fungus was isolated which proved to be markedly different from VM, and since it developed black microsclerotia it was identified as *Verticillium dahliae* Kleb.

An infection of maple by *Verticillium* was first reported in the U.S.A. by Rankin (1914), who observed the disease on hard maples near New York and attributed it to a species of *Acrostalagmus*, which Zimm (1918) included within the genus *Verticillium*, verifying the observations of Rankin. The former successfully inoculated several trees of *Acer saccharum* Marsh, *A. rubrum* L. and *A. platanoides* L. with his pathogen. He further collected for examination from various localities all kinds of maples which appeared to be similarly infected, and proceeded to isolate the effective pathogens. His conclusion was that in all cases this organism differed from the *V. albo-atrum* attacking potato. Since both Zimm and Rankin noted the production by this fungus of sclerotial-like bodies which are typical of *V. dahliae*, but which are not formed by *V. albo-atrum*, it seems probable that the organism concerned was *V. dahliae*. This disease was first reported in Europe by van der Meer (1925, 1926) who stated that wilt of maple was observed at Laren, Holland, in 1919, by the Phytopathological Service and attributed to *V. albo-atrum*. She herself, however, in 1925 isolated *V. dahliae* from 9-year-old specimens of *Acer platanoides* and *A. platanoides* var. *schwedleri* in which plants the trunks, branches and roots showed a characteristic green coloration of the wood. Since 1926 numerous reports have been made of this disease and with but one exception, that of Wollenweber (1929), the identification of a *Verticillium* isolate from maple was based upon its production of such resting structures as dark-walled resting mycelium, microsclerotia and/or chlamydospores, and from the descriptions given the causal pathogen was usually *V. dahliae*. Wollenweber (1929), however, stated that although he considered *V. albo-atrum* was normally a sclerotial fungus he had obtained a few isolations in which, although the mycelium was black, such sclerotia were not developed. These he regarded as constituting a variety which he called var. *caespitosum*. He occasionally isolated a pale form also without black sclerotia which he termed forma *pallens*, but since he did not distinguish between *V. albo-atrum* (the species with resting mycelium), and *V. dahliae* (which produces microsclerotia), his description of *V. albo-atrum* var. *caespitosum* forma *pallens* for what was probably a hyaline variety of *V. dahliae* cannot be maintained.

In the following work investigations into the cultural morphology and physiology and the pathogenicity of VM are described, and a comparison made with the results reported by Isaac (1949) in his work on the hyaline variants of *V. albo-atrum* and *V. dahliae*, respectively, in order to determine whether the strain under examination is either simply a hyaline variant of one of these two species or a distinct type. Attention will also be drawn to the results of cultural studies made of all the hyaline species of *Verticillium* obtainable from the Commonwealth Mycological Institute, Kew, and the Centraalbureau voor Schimmelcultures, Baarn, Holland.

Morphological studies

Twenty-four hours after conidia (approx. $7{\cdot}5 \times 3{\cdot}5\,\mu$) of VM were placed on hanging drops of Dox's agar and exposed to normal daylight at room temperature every one had become swollen, and germ tubes, approximately $100\,\mu$ long and $3\,\mu$ wide at their proximal ends, developed, either one from a single end of each or one from both ends. Six hours later these tubes were $300\,\mu$ long, while after 36 hr. most of them had become branched. After 2 days sporing was prolific, the conidia occurring in clusters at the tips of either verticillately branched conidiophores, or, occasionally, unbranched conidiophores. The fact that spores were formed in abundance must be emphasized since Isaac (1949) reported that conidia were produced only sparingly by hyaline variants of both *V. albo-atrum* and *V. dahliae*.

When VM developed on agar media in Petri dishes was incubated at 25° C. and then examined microscopically the vegetative mycelium was observed to be hyaline and septate, with most of the aerial hyphae aggregated into mycelial strands or 'ropes', very similar to those described for *Gliocladium roseum* by Isaac (1954). These 'ropes' varied from slender ones composed of only three or four hyphae and about 10μ in width, to stout strands composed of large numbers of hyphae and having a diameter of anything up to 100μ, and from them most conidiophores developed, usually from a definite foot cell (Fig. 1). These conidiophores varied in width at the base from approximately 3·3 to 4·9μ and in length from 80 to 200μ and terminated in a whorl of 3 or 4 spindle-shaped phialides approximately 20–43μ in length. Secondary whorls were uncommon, but when occasionally they did occur one of the branches often continued growth and itself eventually became verticillately branched. Each phialide at its apex held a watery vesicle, approximately 5–13·5μ in diameter, into which were abstricted the ovo-cylindrical or fusiform spores approximately 3·5 × 7·5μ.

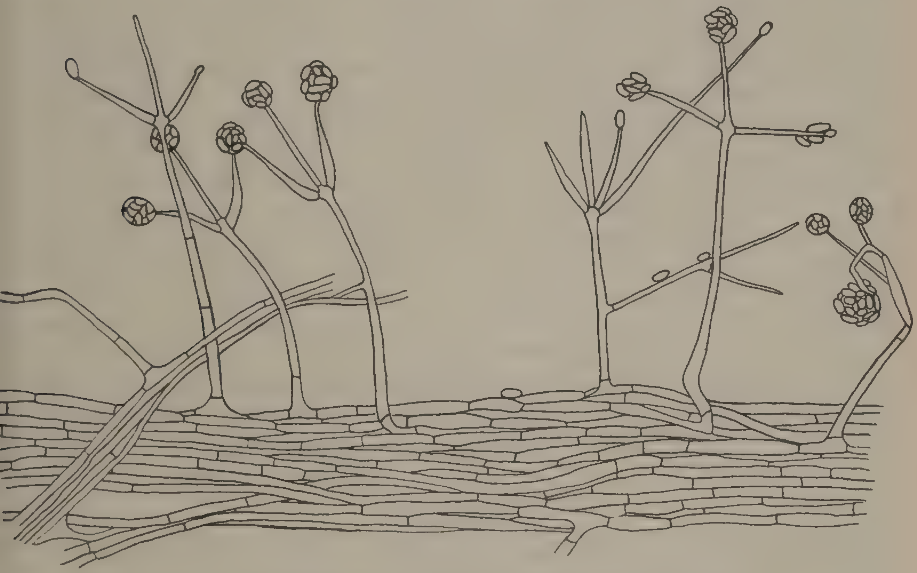

Fig. 1. Conidiophores and a 'rope' of hyphae of VM. (×425.)

In the details of the macroscopic appearance given below VM is described after 10 days' growth, when it had almost completely covered the medium in a 9 cm. Petri dish. The cultures were kept either in the darkness of an incubator at 25° C. or in normal daylight in the laboratory.

In darkness

On Dox's, Czapek's, potato-dextrose, 2·5 and 5% malt-extract agar media VM grew very quickly and consisted of a white floccose mat of aerial and subaerial mycelium with occasional sectors of somewhat prostrate hyphae only. The aerial hyphae were conspicuous since they were usually organized into upstanding mycelial strands or 'ropes', but after about a month many of these had collapsed and the fungus had a more uniformly somewhat flat woolly appearance. The undersurface of the colony showed distinct concentric zonations, and, whereas on Dox's, 2·5 and 5% malt-extract media it appeared white, on Czapek's it was a pale orange colour, while on potato-dextrose agar a deep yellow-green appearance developed where the fungus was in contact with the medium. On sugar-beet-extract agar growth was more rapid than on Dox's and the mycelial 'ropes' tended to be coarser and were conspicuous from the earliest development of the colony, the centre of which was usually upraised. The undersurface was white and no zonation was visible. On oatmeal-extract agar the centre of the colony consisted of fine woolly mycelium, while towards the periphery aerial mycelial 'ropes' were well developed. Sectors consisting of flatter subaerial mycelium were frequent. A slight yellowing was apparent in the prostrate mycelium and no zonations were visible. On prune-extract agar growth was not as luxuriant as on the other media, the colony appearing as a thin plate of subaerial, somewhat woolly mycelium, with indistinct zonations and with a green colour where the fungus made contact with the substrate.

In daylight

VM, grown on Dox's and potato-dextrose agar media in bright summer daylight, developed a deep orange-pink coloration and the concentric zonations were very conspicuous, the individual zones being narrower and their incidence being correspondingly more frequent than when grown in darkness.

In a preliminary investigation into the effects of light upon pigment production VM was grown on Dox's solution in twelve Petri dishes, 30 cm. diameter and 4 cm. deep, each containing 50 ml. of the liquid medium. Four dishes were placed on the laboratory bench where, during the day, they were illuminated by strong summer sunlight, a second four were incubated in darkness at 25° C., while the third set was placed in a light-proof chamber continuously illuminated by a 60 W. electric light bulb approximately 18 in. from the plates. After incubation for 10 days the colonies subjected to the bright sunlight were orange-red in colour, those in the dark were white, while those continuously illuminated by an electric light appeared cream coloured.

These results, together with observations made on the growth of the isolate both in bright summer sunlight and in the more diffuse light of winter, suggest that pigment production ran parallel with increasing light intensity. It must be emphasized, however, that other factors may have contributed to this result, for whereas the temperatures in the incubator and in the light-proof chamber were relatively constant and approximately equal, the colonies kept on the laboratory bench were subject to diurnal fluctuations of temperature, the effects of which have not yet been investigated.

The colour of the pigment was suggestive of either a carotin or a flavone. An extract in acetone gave a negative result when tested for the former, while the fact that it was water-soluble suggested that it might be the latter, but no further tests were made.

When considering the taxonomic status of VM in comparison with established hyaline species of *Verticillium* the morphological details given above are of considerable importance since of all the species received from the C.M.I., Kew and from Baarn, not one produced cultural characteristics identical with those formed by VM. Further, a study of the literature upon these and other hyaline species of *Verticillium*, subcultures of which are now unobtainable, failed to reveal any description of an isolate morphologically similar to that under present investigation. It should be emphasized that among hyaline species of this genus VM is unique in that it alone produces the conspicuous mycelial 'ropes' of aggregated hyphae.

PHYSIOLOGICAL CHARACTERISTICS

The details of the experimental methods employed in the present work were similar to those used by Isaac (1949, 1953, 1954). Usually three or four plates were kept as replicates in each experiment and the average growths for these determined.

Temperature effects

In order to test the effects of temperature upon the growth of VM, and to compare such development with that of the respective hyaline variants of *V. albo-atrum* and *V. dahliae* as reported by Isaac (1949), VM was grown on normal Dox's and potato-dextrose agars and incubated at 4·5, 7·5, 15·0, 20·0, 22·5, 25·0, 27·5, 30·0, 32·5 and 35·0° C., respectively, for 10 days.

Only data for growth on Dox's agar are represented graphically in Fig. 2 since very similar results were obtained on both media.

Wherever growth occurred the appearance of the fungus was normal, and the findings can be briefly summed up in the statement that the optimum temperature for the growth of VM was 25·0° C., it showed some development at 32·5° C. but none at 35·0° C. The lowest temperature at which growth could occur was about 7·5° C.

Since Isaac (1949) found that the hyaline variant of *V. albo-atrum* grew poorly at temperatures of about 25° C. and at 30° C. produced only small yeast-like colonies, and that the optimum temperature for the growth of both this variant and that of *V. dahliae* was 22·5° C., it would appear that on the basis of its reactions to temperatures VM is not a hyaline variant of either of these species.

pH *of media*

The effect of the growth of VM upon various culture media was tested by growing the fungus in an incubator at 25° C. on plates of the following agar media: Dox's, Dox's with 0·2 % ammonium nitrate substituted for the 0·2 % sodium nitrate, Dox's plus sodium acid and potassium acid phosphates, potato extract and potato dextrose. (The initial pH of the two potato and the Dox's plus phosphates media was 6·4, while that of the other two was 5·3.) After 6 hr., then 12 hr. and on each subsequent day for 10 days the pH of plates of each medium was tested by the 'drop' method, using B.D.H. indicators and comparing the results with standards in Clark's (1923) Table.

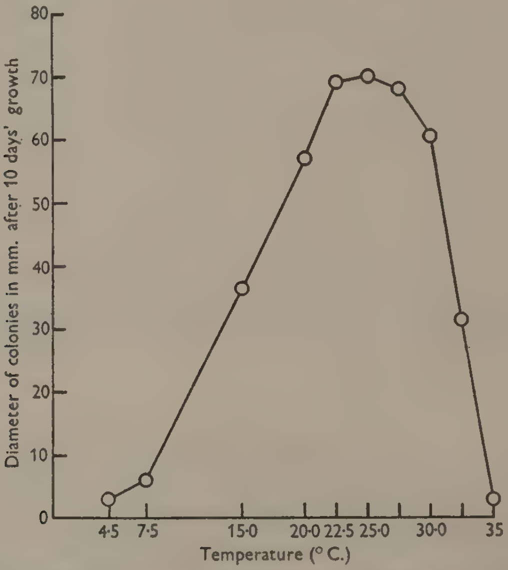

Fig. 2. Influence of temperature upon the growth of VM on Dox's agar medium.

The results indicated that VM was similar to the variants of both *V. albo-atrum* and *V. dahliae* as reported by Isaac (1949), in that it rapidly induced a high degree of alkalinity (pH 8·2) in Dox's and potato-extract media, and acidity (pH 3·2) in the ammonium nitrate medium, but did not alter the pH of potato-dextrose agar. On the other hand, VM differed from these variants in that whereas the Dox's plus phosphates medium remained constant during the development of the latter with VM it rapidly became alkaline (pH 8·0).

When determining the optimum pH for the growth of VM, since the potato-dextrose agar had proved to be naturally buffered, this medium together with normal Dox's was adjusted, as described in earlier papers, to the following pH values: 3·0, 3·6, 4·6, 5·3, 6·4, 7·2, 8·0, 8·6 and 9·6, and three plates of each medium at each pH were inoculated with VM and incubated at 25° C. for 10 days.

Since the fungus showed very similar responses on both media only the results for potato-dextrose agar are illustrated in the graph (Fig. 3).

VM showed normal morphological development on all media of pH 4·6 and over, while on 3·0 it appeared as an irregular, convoluted, waxy looking colony which lacked a normal mycelial development but rather appeared to bud in a yeast-like manner. On Dox's medium of pH 3·6 the fungus was normal since it was, presumably, correcting the acidity of this unbuffered medium during its growth, whereas on potato dextrose of 3·6 the pH value remained constant and the isolate grew rather poorly, appearing as a flat waxy looking plate with radial and concentric furrows and no aerial mycelium.

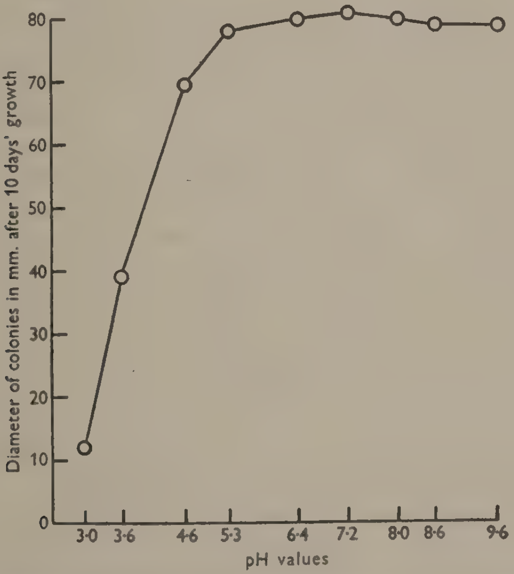

Fig. 3. Influence of initial pH of potato-dextrose agar on the growth of VM.

VM grew well on all media of pH values from 5·3 to 9·6 with an optimum at about 7·2, a value corresponding fairly closely with the best for the hyaline variant of *V. dahliae*, but rather less than the optimum pH for the variant of *V. albo-atrum* which Isaac (1949) stated fell between 8·6 and 9·6.

Variation in the source of carbon

The same sources of carbon and the same percentage of each were used for the growth of VM as were employed by Isaac (1949, 1953, 1954), viz. 1·0, 3·0 and 5·0 % of sucrose, dextrose, maltose and glycerine, respectively, replacing the normal 1·5 % sucrose in Dox's medium. Three plates of each of these twelve media were prepared, inoculated with the fungus and incubated at 25° C. for 10 days. The results are illustrated graphically in Fig. 4.

In general the morphology of VM on all the media was very similar to growth on normal Dox's agar, with a tendency for colonies to produce sectors of rather flat sparsely developed subaerial mycelium most frequently on the sucrose media. The effects of varying the concentration of carbohydrate were not sufficiently consistent to enable any general conclusions to be drawn, but staling effects, appearing as radial and concentric convolutions of the upper and lower surfaces of the colonies, usually became more pronounced with an increase in the percentage of each carbon source.

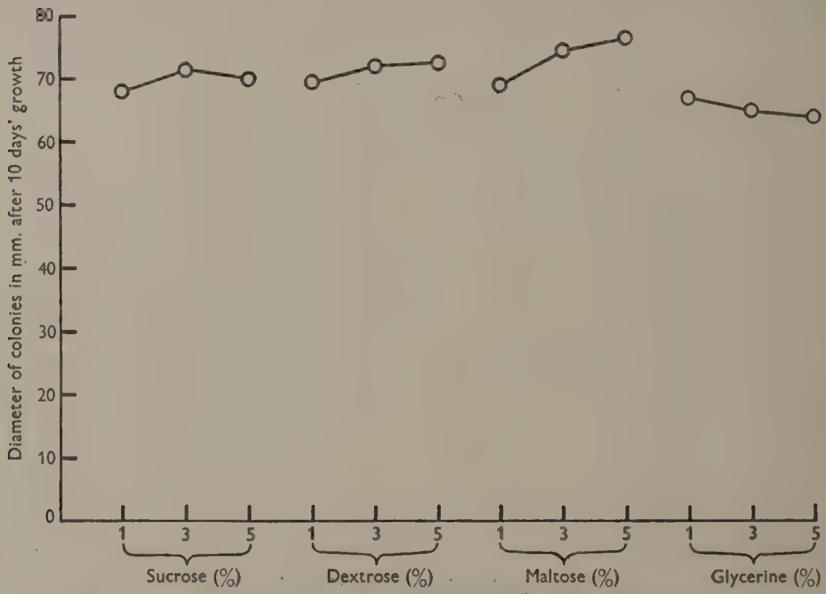

Fig. 4. Influence of the various carbon compounds upon the growth of VM.

In its reactions to these sources of carbon VM resembled very closely the hyaline variants of both *V. albo-atrum* and *V. dahliae* (Isaac, 1949) since all three showed maximum development on maltose, very good growth on sucrose and dextrose and only fair on glycerine. It must be emphasized, however, that the rate of development of VM was considerably faster than that recorded for both the variants.

Variation in the source of nitrogen

In the present work, as in that described by Isaac (1949, 1953, 1954), twenty-one flasks of modified Dox's medium were prepared in which the normal 0·2 % sodium nitrate was replaced, respectively, by 0·1, 0·5 and 1·0 % of asparagine, peptone, sodium nitrate, ammonium nitrate and ammonium sulphate, by 1 % ammonium nitrate buffered with calcium carbonate (0·1, 0·5 and 1·0 %) and by 1·0 % sodium nitrate similarly buffered. Three plates of each medium were inoculated with VM and incubated at 25° C. for 10 days. The results are shown graphically in Fig. 5.

The appearance of the fungus varied considerably with the different sources of nitrogen used.

On the medium containing 0·1 % asparagine growth was profuse, after 10 days the colony consisting of a woolly mat of aerial hyphae much of which occurred in the form of well-developed mycelial 'ropes' radiating outwards from the centre where the hyphae were densely interwoven. On 0·5 % asparagine a dense floccose mat of aerial and subaerial mycelium was developed with indistinct zonations on the undersurface, while on 1·0 % growth was rather poor with only a sparse development of aerial hyphae.

On the 0·1 % peptone medium the colony appeared as a very thin mat of subaerial mycelium of a somewhat waxy appearance, with only a few mycelial 'ropes' near the centre. The undersurface showed distinct concentric zonations. Increasing the concentration of peptone resulted in a more profuse development of VM with conspicuous stout mycelial 'ropes' radiating outwards from the centre of the colony. The upper surface of the fungus on both the higher concentrations was white near the centre and cream-coloured around the periphery and whereas the undersurface on the medium containing 0·5 % peptone was white and distinctly zoned, on 1·0 % it was buff-coloured and no zonations were apparent.

On all the sodium nitrate media the appearance of VM was very similar to that on normal Dox's agar, except that on the lowest concentration the undersurface was somewhat green and with the 1·0 % a rather more dense and tightly floccose colony with a smaller development of mycelial 'ropes' resulted. Buffering with calcium carbonate had no apparent effect upon the morphology of the fungus.

When ammonium sulphate was used as a source of nitrogen the fungus developed as an abnormal yeast-like clump, producing no spores. Buffering this medium with calcium carbonate resulted in the formation of a flat mat of somewhat woolly mycelium with few mycelial 'ropes'.

Of the concentrations of ammonium nitrate used 1·0 % proved to suit best the growth of VM, but on all three concentrations the colony appeared as a woolly felt-like mat of aerial and subaerial mycelium with the undersurface showing radial furrowing indicative of staling.

VM showed maximum development on the sodium nitrate media, particularly in the presence of the buffering calcium carbonate, as contrasted with the hyaline variants of both *V. albo-atrum* and *V. dahliae* (Isaac, 1949) which grew best on strongly buffered ammonium sulphate media. On the other hand, VM resembled these variants in its reactions on unbuffered ammonium sulphate in that the colonies were small and yeast-like and the media rapidly reached a degree of acidity which inhibited further growth. Another point of difference from Isaac's results lay in the finding that VM grew fairly well on ammonium nitrate and continued growth even after the pH of the medium had dropped to 3·4, as contrasted with a fall to 3·0 followed by a cessation of growth with the two hyaline variants. This suggests that all three strains probably preferred the nitrogen of the ammonium ion to that of the nitrate, but whereas it seems

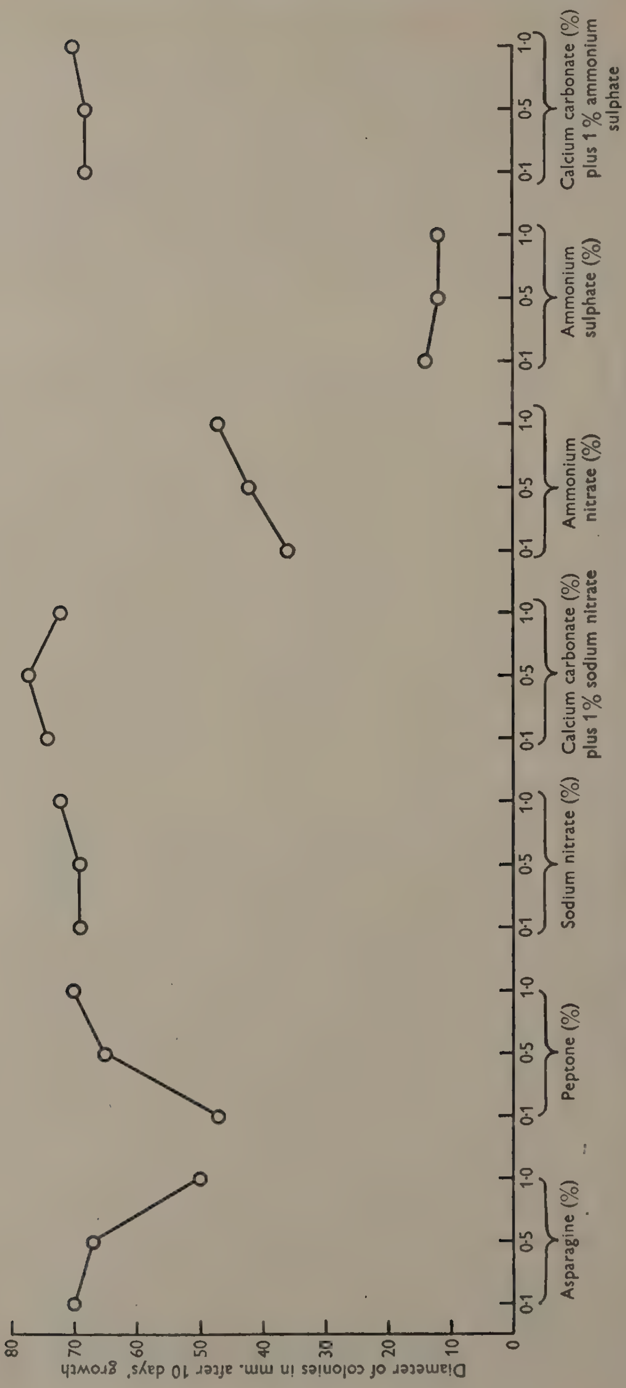

Fig. 5. Influence of the various sources of nitrogen upon the growth of VM.

likely that when the medium became very acid the two variants were unable to use the nitrate, with VM it is not improbable that after the pH had fallen to 3·4, the fungus utilized the nitrate and so continued growth. An increase in the amount of asparagine in the media slowed down growth of VM, as it did also in the case of the hyaline variants reported by Isaac, but with peptone, whereas an increase in concentration was followed by a faster growth of VM, the reverse was true for the other two strains.

STUDIES IN PATHOGENICITY

Since the reported host plant of VM was Japanese maple (*Acer palmatum* Thumb.) the preliminary experiments were designed to attempt to establish the pathogenicity of the fungus to this host. The plants available for inoculation included 4-year-old specimens of both *A. palmatum* and *A. palmatum* var. *purpureum* and 3-year-old seedlings of *A. palmatum* var. *atropurpureum*. Unfortunately, owing to the failure to raise Japanese maple plants from seeds and cuttings, and the comparatively high cost of specimens from nurseries only five of each of those mentioned above were wound-inoculated with VM in the main stem just below ground-level. Three of each variety were similarly inoculated with the strain of *Verticillium dahliae* isolated from the twigs of the infected tree sent from Easthampton, Massachusetts. Suitable control plants which had been wounded only were also kept under observation.

The results of these tests showed that not one of the Japanese maples inoculated with VM developed disease symptoms, whereas all those inoculated with *V. dahliae* began to wilt within 5 weeks. The first signs of disease in these latter were observed during a hot spell in August when the leaves began to show slight crinkling. As the disease progressed in *A. palmatum* and *A. palmatum* var. *purpureum* the leaves wilted, withered, turned brown and fell prematurely, in the former the symptoms developing unilaterally, while in the latter the apical region of the plant was first affected, the symptoms becoming unilateral later. *A. palmatum* var. *atropurpureum* showed only mild symptoms of the disease, consisting of simply a withering of the leaf-tips. In all these infected plants small patches of green discoloration were observed in the wood of the current season's growth and from all of them *V. dahliae* was re-isolated.

Although VM had induced neither external disease symptoms nor internal discoloration of the wood, it was re-isolated, in its original morphological form, from five of the Japanese maples, but only from the wounded regions. This suggests that VM is either a very weak pathogen, or, more probably, that it had survived as a saprophyte in the tissues killed during wounding.

All the control plants remained healthy. From this evidence it would appear probable that the wilt disease of the Japanese maple at Easthampton, Massachusetts, was induced by a strain of *V. dahliae*, and that VM was present as a secondary infection, either as a saprophyte or as a mild pathogen.

In order to determine whether VM could be classified on pathological criteria a further series of inoculation tests were next conducted involving

VM, the *V. dahliae* from Japanese maple and a hyaline variant of this latter, which had arisen in artificial culture, as possible pathogens, and potato (Arran Banner), hop, strawberry (Giant Huxley), tomato (Kondine Red), eggplant, sainfoin, cucumber, antirrhinum, tobacco and lupin as possible hosts, since all these are known to be susceptible to infection by various species of *Verticillium*. Series of five or seven of these plants were wound-inoculated just below ground-level, with suitable control plants, with wounds only, being kept for each set of experiments.

At the onset of well-developed wilt symptoms or after natural 'die-back' in autumn, efforts were made to re-isolate the inoculated fungi.

The results indicated that VM was not pathogenic to any of the plants tested, while both *V. dahliae* and its hyaline variant induced disease symptoms only in potato, antirrhinum and tomato, from which wilted plants the pathogens were re-isolated, the re-isolate being, in each case, morphologically identical with the original inoculum.

All the control plants remained healthy.

The fact that VM was not pathogenic to the plants listed as usually susceptible to both *V. albo-atrum* and *V. dahliae* and the knowledge that the hyaline variants of both these species either retain their full pathogenicity or become only a little less pathogenic than the parent type (Isaac, 1949), leads to the conclusion that VM is a hyaline variant of neither *V. dahliae* nor *V. albo-atrum*. Also it is worthy of note that since *V. dahliae* from maple and its hyaline variant induced symptoms in only relatively few of the host plants tested, this isolate is probably a distinct physiological strain with a somewhat restricted range of pathogenicity.

Conclusions and discussion

The morphological differences between the strain VM and the respective hyaline variants of *V. albo-atrum* and *V. dahliae* and the other established hyaline species of *Verticillium* in culture have already been discussed. It must be emphasized here that VM remained constant throughout the present investigation; it alone had a fluccose appearance on agar media due to the aggregation of aerial hyphae into mycelial 'ropes', and it was also unique in that it produced an orange to pink pigment in daylight. The further work on the physiology and pathogenicity of VM, together with the data reported by Isaac (1949) for the hyaline variants of *V. albo-atrum* and *V. dahliae*, are briefly summarized in Table 1.

It must follow from a consideration of this accumulated evidence that VM is distinct on morphological, physiological and phytopathological grounds. The question now arises as to what rank should be accorded to this strain. The absence of sex organs to be used as a basis of classification makes the question of status largely one of personal opinion and interpretation, but since Isaac (1949, 1953) suggested that the principle of convenience is the ultimate criterion of classification, and, since he argued from this that specific rank be ascribed to the strains of *Verticillium* he was then studying, we are now forced to accord a similar status to the organism under present examination and we propose naming it *Verticillium intertextum* sp.nov.

Table 1. *Comparison of VM and the hyaline variants of* V. albo-atrum (*Vaa.h.*) *and* V. dahliae (*Vd.h.*) *respectively*

	VM	Vaa.h.	Vd.h.
Optimum temperature for growth (° C.)	25·0	22·5	22·5
Growth in culture at 30° C.	Fairly good	Nil	Fairly good
Optimum pH for growth	7·2	8·0–8·6	5·3–7·2
Best source of carbon	Maltose	Maltose	Maltose
Growth on unbuffered ammonium nitrate medium	Fairly good	Nil	Nil
Growth on unbuffered ammonium sulphate medium	Nil	Nil	Nil
Best source of nitrogen	Sodium nitrate when buffered with calc. carbonate	Ammonium sulphate when buffered with calc. carbonate	Ammonium sulphate when buffered with calc. carbonate
Host range	Re-isolated from *Acer palmatum* only. Induced no wilt symptoms in any plant tested	Wide	Wide

Verticillium intertextum sp.nov. (Fig. 1)

Mycelium septatum in luce flavum, in tenebris album. Mycelium elevatum aggregatum in filis. Hyphae fertiles verticillate ramosi plerumque ex filis excrescentes cum apicale conidiorum, globo hyalinorum et unicellularium oviformium, 3·5–12·0μ longorum × 2·5–4·5μ crassorum, av. 7·45 × 3·45μ.

Hab. Acer palmatum. Prope Easthampton, Massachusetts, U.S.A. 1950.

Type material of this new species will be deposited at the Commonwealth Mycological Institute, Kew; in the Herbarium, Royal Botanic Gardens, Kew; and in the Centraalbureau voor Schimmelcultures, Baarn, Holland.

Our thanks are due to Prof. F. A. Mockeridge for advice in the preparation of the manuscript, to Dr A. G. Hamilton for providing research facilities at St Thomas's Hospital, London, to Mr S. Usher for help with the Latin diagnosis and to various research and mycological institutes for kindly providing some of the fungi used.

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AQUATIC ASCOMYCETES: FURTHER SPECIES FROM THE ENGLISH LAKE DISTRICT

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(With 9 Text-figures)

Belonidium rhenopalaticum Rehm, *Pleospora scirpicola* (DC.) Karst., *Rebentischia* sp., *Physalospora aquatica* sp.nov., and *Wettsteinina niesslii* Müller are described as occurring submerged on dead reed-swamp plants in the English Lake District. The occurrence there of *Leptosphaeria lemaneae* (Cohn & Woron.) Sacc. is also noted. The flora of freshwater Ascomycetes is briefly discussed.

Belonidium rhenopalaticum Rehm

In a recent paper (Ingold, 1954) two Discomycetes, *Belonium excelsius* (Karst.) Boud. and *Niptera pulla* (Phill. & Keith) Boud., have been noted as abundant species on submerged, dead, *in situ* stalks of *Phragmites* in lakes of the English Lake District. Observations made in Windermere and Esthwaite during the summers of 1953 and 1954 indicated that, there at least, a third species is very often present. This has been identified as *Belonidium rhenopalaticum* Rehm and is easily confused with *Belonium excelsius* in the field, although microscopic examination allows the two species to be separated on the basis of several clear-cut characters.

Like *B. excelsius*, *Belonidium rhenopalaticum* has conspicuous whitish apothecia 1–3 mm. in diameter with the excipulum dark brown or almost black. In general, however, the surface of the hymenium is flat in the latter species, whereas it tends to be convex in the former. This is the only character that I have been able to use in the field to distinguish the two species, but it is not always reliable. Attention has been drawn to the fact (Ingold, 1954) that apothecia of *Belonium excelsius* are most often to be found on the lowest internodes of submerged attached stalks of *Phragmites*. The same is true of *Belonidium rhenopalaticum*. An examination of a random collection of fifty-two stalks bearing apothecia from a small colony of *Phragmites* near the inflow end of Esthwaite showed that thirty-six bore apothecia of *Belonidium rhenopalaticum* and the remaining sixteen those of *Belonium excelsius*. A particular stalk appeared to bear only one of the two species.

The structure of the apothecium is illustrated in Fig. 1. Although sessile it has a definite stalk as in *B. excelsius*. The excipulum is pseudoparenchymatous, and its outermost cells have dark walls. Between this tissue and the hymenium and subhymenium is a zone of fine interwoven hyphae. Both this tissue and the pseudoparenchymatous excipulum have numerous conspicuous interhyphal crystals, usually grouped into rosettes, of what is probably calcium oxalate, since they are insoluble in acetic but soluble in hydrochloric acid. It might be argued that such crystals are of little systematic importance and may owe their origin to the peculiarities of the habitat. However, it is worth noting that, although growing in precisely the same ecological niche, neither *Belonium excelsius* nor *Niptera pulla* bear crystals. From the surface cells of the apothecium, especially in the more basal regions, unbranched brown septate hyphae usually grow out. Most of them represent anchoring hyphae, their free ends making contact with the surface of the reed. They are particularly

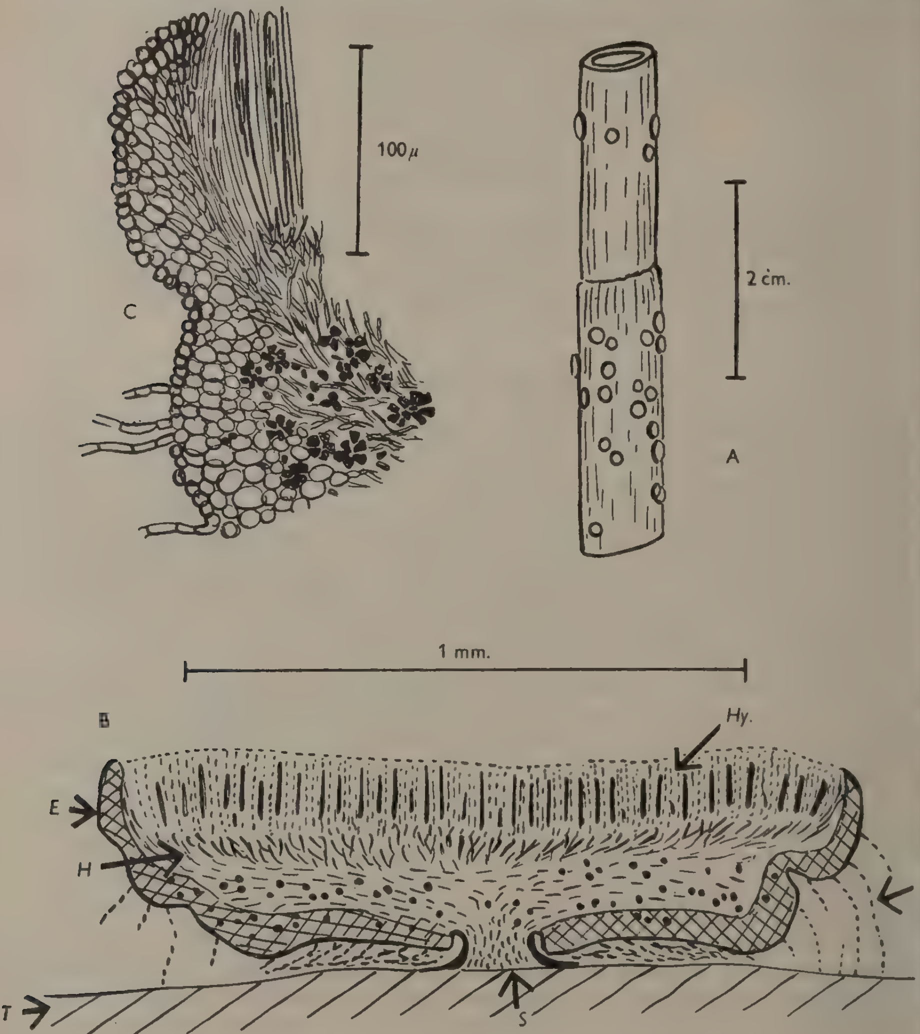

Fig. 1. *Belonidium rhenopalaticum*. A, portion of *Phragmites* stalk with apothecia. B, median section through apothecium; *T*, reed tissue; *S*, stalk; *E*, excipulum; *H*, hypothecium; *Hy*., hymenium; *A*, anchoring hairs. The black dots represent the larger rosettes of crystals. C, part of apothecium (shown in B) more highly magnified. The crystals are shown black.

numerous in the confined space between the lower surface of the apothecium and the substratum. In some collections made from Windermere and in one from Lough Neagh in Northern Ireland, these hairs were absent.

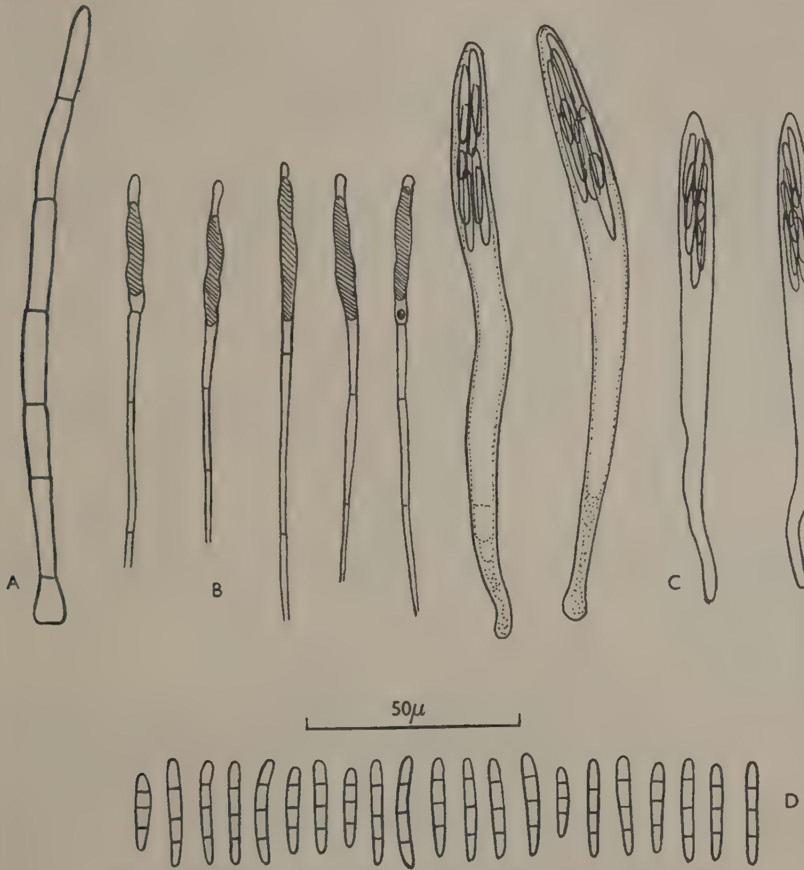

Fig. 2. *Belonidium rhenopalaticum*. A, hair from surface of apothecium. B, paraphyses. C, asci. D, ascospores.

Mature asci are 110–140 × 8–12 μ and are somewhat pointed. Each contains eight spores. A random sample of fifty freshly discharged spores averaged 24·2 × 3·1 μ (extremes 16–32 × 2–4 μ). The spores are colourless and usually 3-septate. Paraphyses are simple. The end cell of each is 30–50 × 3–4 μ and the greater part of it is filled with colourless oil. The outline of this end cell tends to be somewhat wavy (Fig. 2).

In describing *Belonidium rhenopalaticum*, Rehm (1896) cited as type material: Exsicc. Moug. et Nestl. Stirp. vog. 983 (sub *Eustegia arundinacea* Fr.). I have examined this type in the British Museum (Nat. Hist.) and, although it is not in very good condition, I have little doubt that the Lake District collections are properly assigned to this species. Twenty spores of the type taken at random in a lactophenol mount averaged 21·6 × 2·2 μ (extremes 17–30 × 1·4–2·7 μ). This agrees well with Rehm's figures (18–24 × 3 μ) and with the Esthwaite material (24 × 3 μ). Further, the type material has brown simple hairs like those seen in the fresh material and, above all, the apothecial tissue contains the same kind of crystals.

It should be noted that *Belonidium mediellum* (Karst.) Rehm, also on *Phragmites*, has 4-celled spores 21–25 × 2–2·5 μ and would seem to be very close to *Belonidium rhenopalaticum*. It is quite possible that the two species are really the same. However, I have been unable to examine type material of *B. mediellum*.

The three Discomycetes commonly found on submerged *Phragmites* in the English Lake District are readily distinguishable by a number of characters. They can, however, be separated on spore-size alone as shown in Table 1.

Table 1. *Ascospore size of* Belonium excelsius, Belonidium rhenopalaticum *and* Niptera pulla

	Measurement in μ of fifty freshly discharged spores selected at random	
Species	Average	Range
Belonium excelsius	70·6 × 3·2	52·5–92·5 × 2·1–5·0
Belonidium rhenopalaticum	24·2 × 3·1	16–32 × 2–4
*Niptera pulla**	35·8 × 4·2	31–40 × 3·8–5·0

* Measurements made from a collection of *N. pulla* on submerged *Phragmites*. The closely similar measurements given earlier (Ingold, 1954) refer to a collection from *Phalaris*.

Pleospora scirpicola (DC.) Karst.

This fungus was found commonly on submerged, dead, but still attached, stalks of *Schoenoplectus lacustris* in Blelham Tarn. It was reported as an aquatic species nearly a century ago by Pringsheim (1858). In Blelham Tarn it occurred usually 10–30 cm. below normal lake level. By leaning over the side of a boat and by feeling dead submerged reeds between finger and thumb this fungus can be detected because the ostioles project slightly beyond the surface and so are felt as gritty particles. A submerged species of *Metasphaeria* is also frequently present on *Schoenoplectus*, but, as the ostioles do not project, it cannot be detected by touch.

The perithecia (or more correctly pseudothecia) are black and about 0·2 mm. in diameter. Each contains only a few ripe asci (Fig. 3 A). These are large and ovoid when unexpanded. However, the ascus is bitunicate and just before discharge the inner layer expands considerably (Fig. 3 B). The spores are large (57–77 × 23–28 μ), muriform, with five primary septa, and are colourless or faintly yellow because of pigmentation of the oil which occurs as numerous small droplets in the cells of the spore (Fig. 3 B). An interesting feature is the mucilage sheath around the spore (Fig. 3 C). This is difficult to see when the spore is still within the ascus. Immediately following discharge in water the sheath may be seen to consist of an inner and an outer region, the former being delimited by a definite membrane. In the course of a few minutes following discharge the mucilage sheath rapidly increases particularly in length, no doubt following absorption of water. These enlarged sheaths can be seen clearly when spores are mounted in indian ink. The whole somewhat slug-like structure may be nearly a millimetre long. It is tempting to suggest that this extraordinary mucilage sheath may have a biological value, perhaps in anchoring the spore to a suitable substratum.

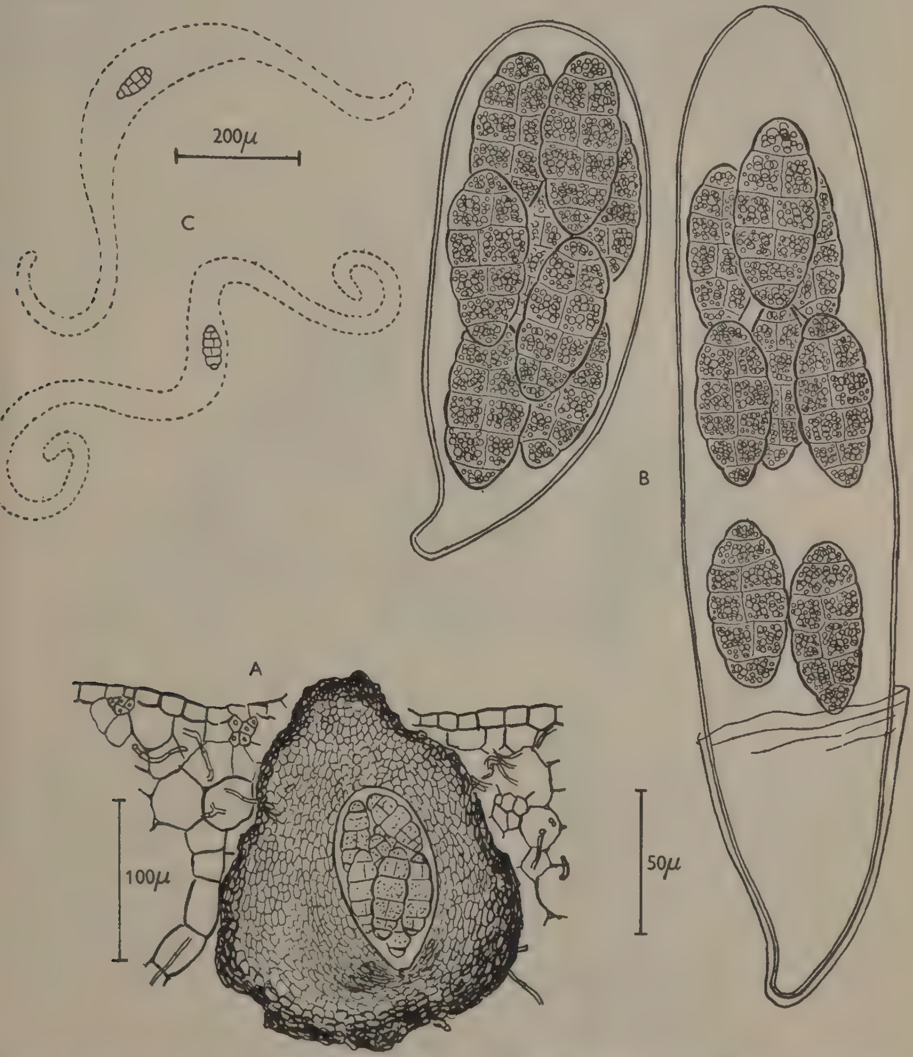

Fig. 3. *Pleospora scirpicola*. A, median longitudinal section through a pseudothecium; the ostiole has not yet developed. B, expanded ascus and unexpanded ascus. C, freshly discharged spores showing the mucilaginous sheaths.

Müller (1951) would transfer this species to *Pyrenophora*, but Wehmeyer (1954) considers that it should remain in *Pleospora*.

Rebentischia sp. on *Phragmites*

A species of *Rebentischia* has been found on several occasions in Little Langdale Tarn and in Esthwaite, completely submerged on dead, attached stalks of *Phragmites communis*. The black perithecia are rather large (0·6–1·2 × 0·6–0·8 mm.) and usually somewhat oval (Fig. 4A). A section is

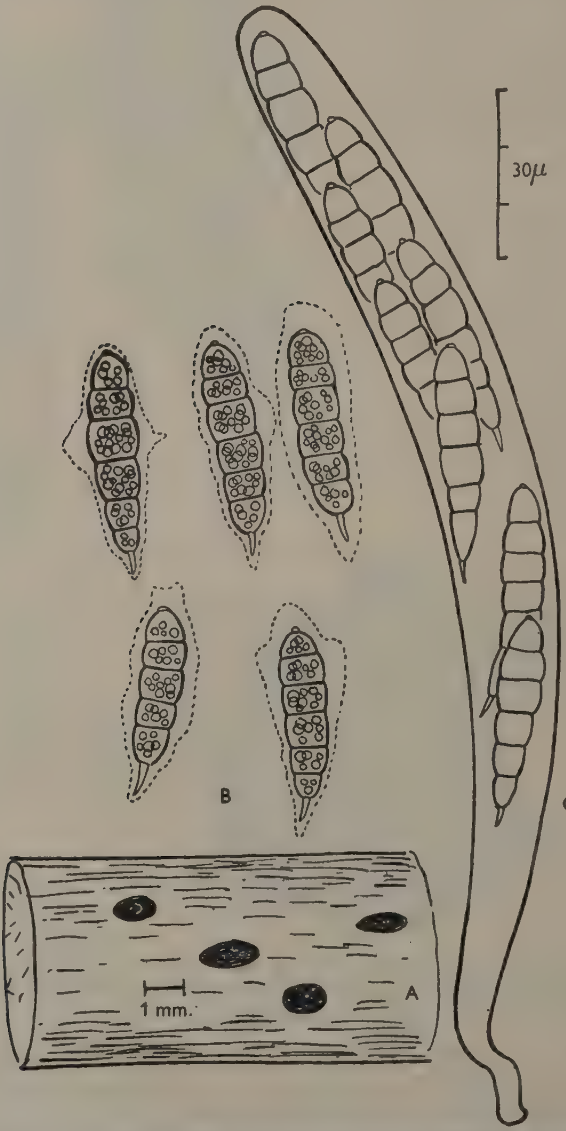

Fig. 4. *Rebentischia* sp. A, portion of *Phragmites* stem bearing perithecia. B, freshly discharged spores each surrounded by a mucilaginous sheath. C, an unexpanded ascus.

shown in Fig. 5. The ostiole is probably not as wide as figured. No doubt during the preparation of the section the two sides of the perithecium have become dragged apart to some extent. It will be seen that the perithecium is essentially subepidermal, although a number of cells from the outmost layers of the cortex of the reed may become incorporated in the black perithecial wall. An interesting feature is that this black wall is missing in the basal region of the perithecium where only the lining layer of thin-walled hyphae occurs from which the asci and paraphysoid tissue arise. The asci are of the normal bitunicate type of *Leptosphaeria* and its allies. The spores (35–42 (including the tail) × 6–8 μ) are shown in Fig. 4. Each is rust brown, of five to six cells and with a minute curved tail which distinguishes *Rebentischia* from *Leptosphaeria*. On mounting freshly discharged spores in indian ink they may be seen to be surrounded by an irregular mucilage sheath.

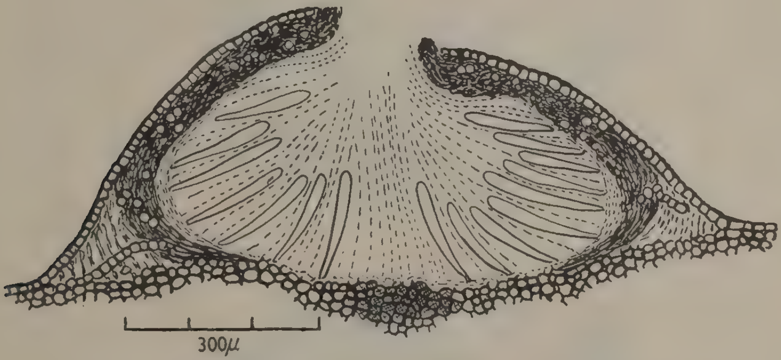

Fig. 5. *Rebentischia* sp. Median longitudinal section of pseudothecium.

This fungus agrees very well with a species of *Rebentischia* described by Müller (1950) from dead *Phragmites* stalks in Switzerland and identified by him as *Rebentischia typhae* Fabre. However, the fungus described by Fabre (1880) on *Typha angustifolia* had yellow spores and the tail-cell was very much larger than in the spores figured by Müller and by me (Fig. 4). There seems some doubt in identifying the fungus as *Rebentischia typhae*. Material of the Lake District collection is deposited at the Commonwealth Mycological Institute (Herb. I.M.I. no. 45889).

Physalospora aquatica sp.nov. (Figs. 6, 7)

Perithecia nigra, globosa, 80–120 μ diam., in substrato immersa. Asci unitunicati, ampulliformis, 70–90 × 20–25 μ. Ascosporae non-septatae, hyalinae, ellipsoido-fusiformis, 15–25 × 5·5–7·5 μ, tenuibus parietibus, olei guttulas multas continentis.

Hab. In caulibus emortuis submersis *Eleocharis palustris*, *Schoenoplecti lacustris*, *Equiseti fluviatilis* et *Typhae angustifoliae*, Moss Eccles Tarn, Lancashire, Anglia. Typus: Herb. I.M.I. 51811 (*Eleocharis palustris*).

This minute species was found in considerable abundance on permanently submerged leaf-bases and stalks of reed-swamp plants including

Eleocharis palustris, *Equisetum fluviatile*, *Schoenoplectus lacustris* and *Typha angustifolia* from various lakes and tarns in the English Lake District.

The perithecia are black, nearly spherical, minute (80–120 μ diam.) and completely immersed in the substratum (Fig. 6). The ripe asci are 70–90 × 20–25 μ, considerably inflated in the middle but much narrower near base and apex, and unitunicate (Fig. 7). The spores are colourless,

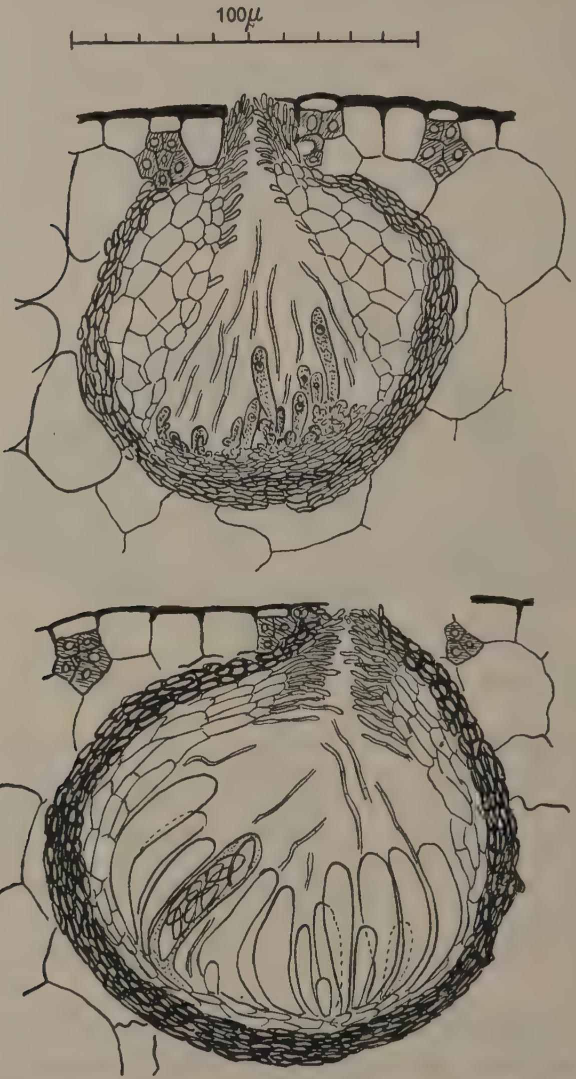

Fig. 6. *Physalospora aquatica* on *Eleocharis palustris*. Median longitudinal sections of perithecia. Above, a young perithecium; below, a mature one, the contents of only one ascus being shown.

ellipsoid-fusiform, 18·7 (extremes 15 and 25μ) × 6·5μ (extremes 5·5 and 7·5μ).

The spore wall is very thin and the spore is closely packed with small oil droplets except for a clear region which seems to represent the nucleus. Dr J. A. von Arx has kindly examined a sample of my material and, although he considers that it is close to the type species *Physalospora alpestris* Niessl, he suggests that it should be placed in a separate species. In *P. alpestris*, although the spore size is much the same as in the aquatic species, the spore shape is different, the ends being broadly rounded.

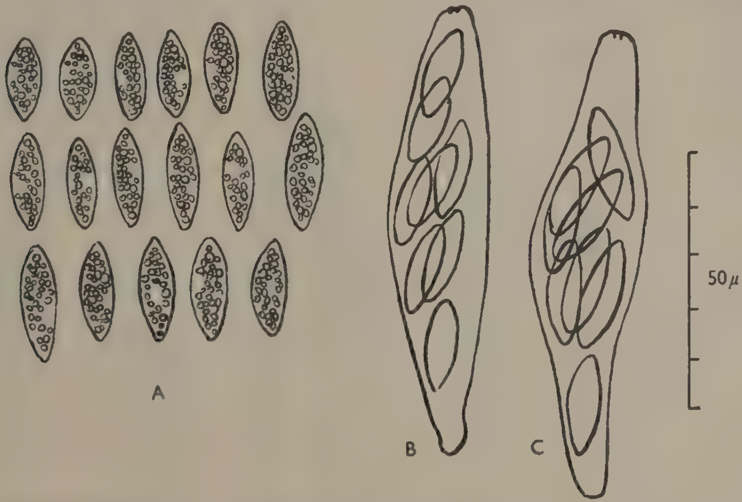

Fig. 7. *Physalospora aquatica.* A, freshly discharged ascospores. B, ascus. C, ascus. A and B from collection on *Eleocharis palustris*, C from *Equisetum fluviatile*.

Wettsteinina niesslii Müller

This species has been collected frequently on submerged, dead, but still attached stalks of *Phragmites communis* in Esthwaite, Blelham Tarn and Windermere.

The black perithecia occur usually grouped together and normally fairly close to a node of the reed (Fig. 8A). The ascus is bitunicate (Fig. 8B). When its outer wall bursts rupture usually takes place so that a little cap of this wall is carried up on the apex of the expanded inner wall. The ascus contains eight spores 40–60 × 7–10μ (average 51·0 × 8·9μ). Each is uniseptate, colourless and both its cells contain numerous small oil globules. The spore is furnished with a sheath of mucilage which fits tightly around its middle region, but projects considerably at the two ends to a distance of up to 40μ. In spores just after discharge the projecting ends of the sheath are much shorter but rapidly increase to full length by the absorption of water. In *Wettsteinina niesslii* (Müller, 1950) the spores are pale yellow when mature and 3-septate. In all the collections I have examined freshly discharged spores are colourless and 1-septate. However, the failure to become pigmented and to develop further septa may well be

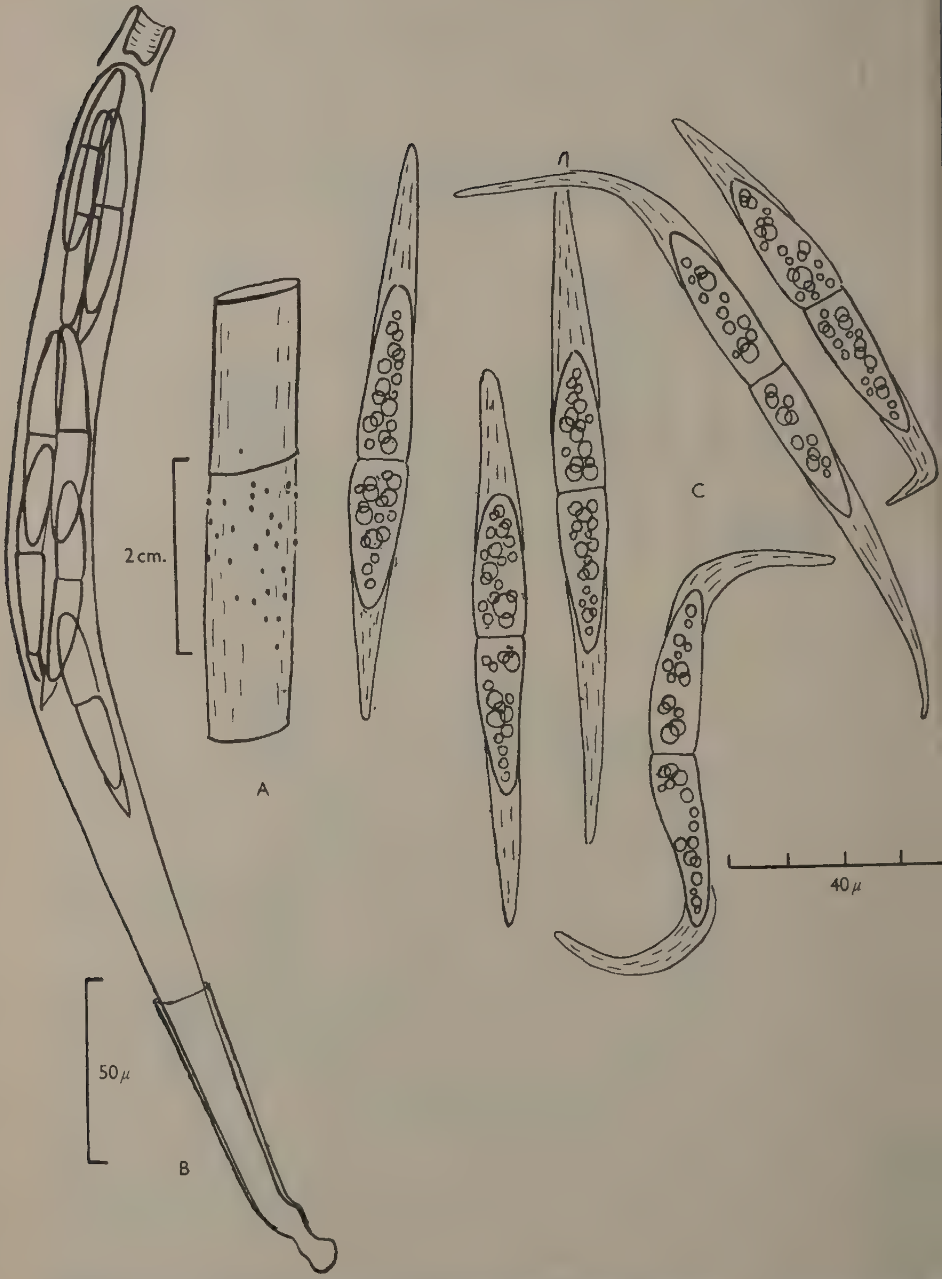

Fig. 8. *Wettsteinina niesslii.* A, portion of *Phragmites* stalk bearing perithecia. B, expanded ascus. C, freshly discharged ascospores.

connected with the aquatic habit. I have not found this fungus on *Phragmites* except in a permanently submerged position. There seems little doubt that it is correctly assigned to *Wettsteinina niesslii.* Dr Müller has examined my drawings and agrees with the identification. Material is deposited at the Commonwealth Mycological Institute (Herb. I.M.I. no. 51812).

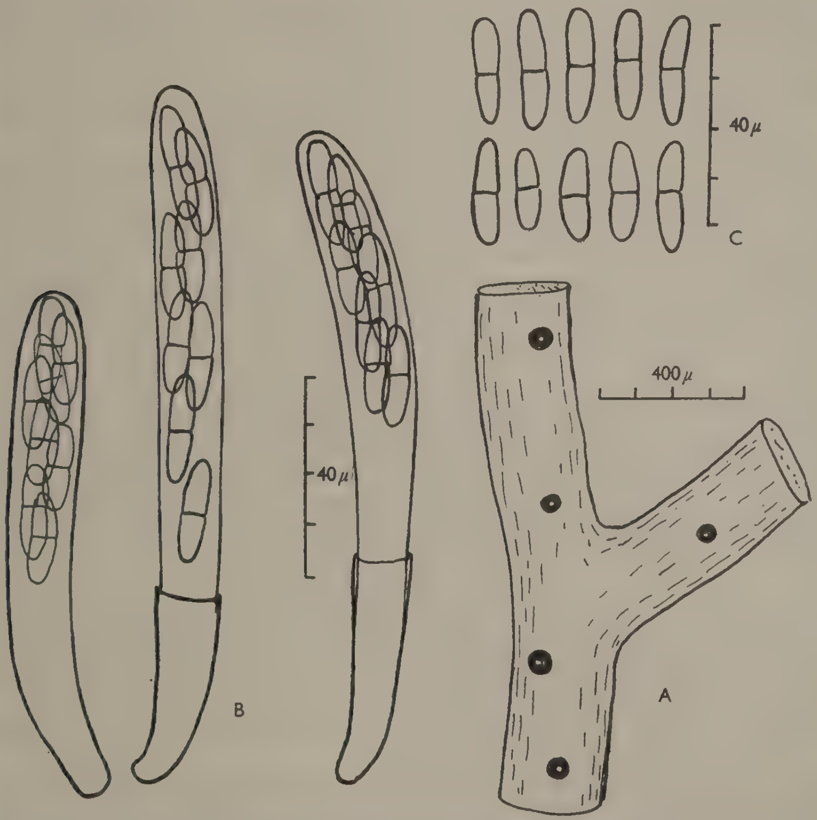

Fig. 9. *Leptosphaeria lemaneae.* A, portion of the thallus of *Lemanea* bearing five pseudothecia. B, three asci, two of which are expanded. C, freshly discharged spores.

Leptosphaeria lemaneae (Cohn & Woron.) Sacc.

This species was found abundantly on the alga *Lemanea* collected in Stock Gill at Ambleside and Brathay River at Clappersgate. It has already been described fairly fully from British material (Brierley, 1913). The black perithecia are very minute (60–80 μ dia.). The asci are bitunicate (Fig. 9). The spores are 18–23 × 5–6 μ, colourless and, in my experience, usually two-celled, although after liberation and before germination two additional septa may appear.

Discussion

In this and in previous papers (Ingold, 1951; Ingold & Chapman, 1952; Ingold, 1954) some of the aquatic Ascomycetes of the English Lake District have been described.

So far as Discomycetes are concerned my collections suggest that not many more species remain to be recorded. In my recent paper (1954) I failed, however, to mention *Vibrissea truncorum* (Alb. & Schw.) Fr. which is common, usually submerged, on sticks in streams in the Lake District and also occasionally in the lakes themselves.

With regard to Pyrenomycetes the position is very different. I have considered only a minority and many more await study.

It should be emphasized that Ascomycetes contribute largely to the aquatic fungus flora of lakes bordered by reed-swamps. What is needed now is an intensive study of these fungi by a mycologist at a freshwater biological station, so that a reasonably complete picture of this flora can be built up and so that the occurrence of these fungi throughout the year, their relation to the reed-swamp plants and their tolerance of different types of water can be fully investigated.

My thanks are due to Mr Carey Gilson for permission to work from time to time at the Freshwater Biological Association's laboratories on Windermere, to Prof. E. H. Warmington for help with the Latin diagnosis, to various mycological friends who have given me valuable advice, and to the Central Research Fund of the University of London for a grant towards the expenses involved in this work.

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PROCEEDINGS, 1954

MEETINGS

9 January 1954. Meeting held at the London School of Hygiene and Tropical Medicine, London, W.C. 1, at 11.0 a.m. The President, Dr S. D. Garrett, in the Chair.

J. A. Tomlinson. Observations on the behaviour of zoospores of *Spongospora subterranea* and *Olpidium brassicae*.

See *Tijdschr. PlZiekt.* **60**, 1–65, 135–137.

Mrs P. M. Fleetwood Walker. Observations on the physiology of *Phytophthora fragariae*.

A nutritional comparison, using liquid cultures, was made between *Phytophthora fragariae*, the cause of Red Core disease of strawberries, and the relatively unspecialized parasite *P. cactorum*. In a basal medium containing magnesium sulphate and potassium dihydrogen phosphate both fungi showed similar qualitative responses to a range of carbon sources and both were heterotrophic for thiamin, though the requirement of *P. fragariae* for this vitamin was much greater than that of *P. cactorum*. In the same basal medium, with dextrose as a carbon source, *P. cactorum* grew on inorganic and simple organic nitrogen compounds, while *P. fragariae* grew only on hydrolysed casein. *P. fragariae* did not grow, however, in a medium in which the hydrolysed casein was replaced by an equivalent mixture of eighteen amino-acids, either as DL- or L-forms. Hydrolysed casein was, therefore, examined chromatographically in an attempt to isolate an active factor for *P. fragariae* growth. These experiments led to the discovery that the papers used in the chromatographic analyses themselves contained an inorganic factor with growth activity for *P. fragariae* in the synthetic amino-acid medium. Of nine elements whose presence was demonstrated in the chromatography paper ash by spectrographic methods, only calcium promoted growth of *P. fragariae* in the synthetic amino-acid medium. Maximum growth of the fungus was produced with a calcium concentration of 70 mg./l.

Miss M. Gregg. Oospore production in pure cultures of *Phytophthora fragariae*.

Phytophthora fragariae produces oospores quite freely in roots of infected strawberry plants but less readily in pure culture on artificial media. An examination of three isolates, which produce oospores on French bean and oat agar media at 20° C., showed that in almost every instance the oogonia were formed in contact with intact pieces of bean or oat tissue present in the medium.

In experiments with artificial membranes there was evidence that contact may act as a stimulus to sexual reproduction in this fungus. Membranes composed of 'Cellophane', celloidin, and mixtures of paraffin wax and 'Vaseline' were investigated. Sterile membranes were placed on the surface of inoculated French bean agar plate cultures and covered with a second layer of agar medium. Where hyphae made contact with the membrane appressoria developed, and hyphae arising from these appressoria penetrated the membrane. Following the production of appressoria, oogonia and antheridia developed in close association with them.

The number and size of the reproductive organs formed varied with the type of membrane. Particularly large oogonia and oospores, diameter 33–83μ and 23–60μ, respectively, were produced in contact with 'Cellophane' membranes. Celloidin membranes were less easily penetrated than those of 'Cellophane' by hyphae of *P. fragariae*. Furthermore, penetration and reproduction only occurred in contact with very thin membranes of celloidin. Oogonia and oospores formed were smaller than those on 'Cellophane' (diameters 28–42 μ and 20–35 μ, respectively). Soaking the celloidin membranes in 99% alcohol for 24 hr. before autoclaving rendered them more easily penetrable by the fungus. The number and size of oogonia and oospores produced in contact with these treated membranes were similar to those formed on 'Cellophane'.

In the case of 'Vaseline'-paraffin wax membranes, reproduction depended upon the relative proportions of wax and 'Vaseline' in the membrane, but where reproduction occurred little variation in size of oogonia was evident.

H. Meier and J. Webster. Electron microscope observations on zoospore cysts in the Saprolegniaceae.

See *J. exp. Bot.* **5**, 401–409.

A. D. Greenwood. Some physiological aspects of asexual reproduction in the Saprolegniales.

Asexual reproduction in *Saprolegnia ferax* was induced by transferring mycelium from pea-meal broth to water. At 25° C. zoospore liberation commenced about 2 hr. later following a well-defined sequence of stages. The process was illustrated by photographs and a ciné film as the basis for discussion of the physiology of experimentally accessible parts of the process which are under investigation.

Sporangium growth: 60–90 min. Involves changes in form and growth rate of the hyphal tips which generate the club-shaped sporangia. Terminated by rapid insertion of the basal cross-wall in the final 2–4 min.

Production of spore initials: 15–30 min. The cytoplasm becomes dissected into spore initials by narrow clefts which develop outwards from the central vacuole. Concludes dramatically in a climax of about 30 sec. duration marked by sudden loss of turgor and obliteration of the central vacuole accompanied by movements of the basal wall and apical papilla and the swelling of the spore initials. The significance of these events was discussed.

Spore maturation: approximately 15 min. The spores contract, take their normal form; moving cilia and contractile vacuoles become visible. Finally they can be seen rocking and rotating, separate from each other and from the containing sporangium.

Dehiscence: within the next 30 min. the spores emerge. The mode of opening of the exit papilla and the factors concerned in spore discharge were considered. Retardation of the process by low concentrations of sodium chloride was described.

20 March 1954. Meeting held at Birkbeck College, London, W.C. 1, at 2.0 p.m. The Vice-President, Prof. C. T. Ingold, in the Chair.

G. C. Ainsworth. The pattern of taxonomic publication.

See *Taxon*, **3**, 77–79, 1954.

The paper was followed by a Conversazione in which over forty demonstrations were exhibited.

26 June 1954. Meeting held in the Botany School, Cambridge, at 9.30 a.m. The President, Dr S. D. Garrett, in the Chair.

C. C. V. Batts. Observations on infection of wheat by *Ustilago tritici*.

Since the life history of *Ustilago tritici* was first described about fifty years ago it has generally been accepted that the fungus enters the ovary by way of the stigma. A few more recent accounts have described penetration through the ovary wall also. The subsequent path of the fungus leading to infection of the embryo proper has received very little attention since the work of Lang in 1910.

In 1953 ears of susceptible wheat varieties were inoculated at anthesis by the partial vacuum method. Ears were fixed at intervals (6, 11, 18, 33 days) after inoculation and the path of infection investigated in more than 60 grains by means of surface preparations or serial microtome sections.

No evidence of passage of the fungus down the style was found; mycelium was never seen at the apex of the grain. Six days after inoculation, spores on the ovary wall (pericarp) had germinated and mycelium was present. After 11 days intracellular mycelium was found in the pericarp. Appressoria were present at the points of entry of the fungus and funnels and sheathing of internal mycelium were seen. After 18 days mycelium had begun to permeate the testa. After about 30 days the fungus had entered the scutellum from the testa on the dorsal side of the grain. This differs greatly from the findings of Lang.

The mycelium developed profusely in the scutellum and entered the embryo proper but did not penetrate the plumule and radicle.

R. LEACH. Recent observations on the *Botrytis* infection of beans.

'Chocolate spot' on leaves of field beans can be caused by either *Botrytis cinerea* or *B. fabae*. The spots caused by *B. cinerea* are inconspicuous compared with those caused by *B. fabae*, frequently only epidermal cells being affected, whereas *B. fabae* nearly always causes necrosis of the mesophyll. From the agricultural viewpoint, chocolate spot disease is that caused by *B. fabae*, since a bean crop even heavily infected by *B. cinerea* looks to be only insignificantly spotted, and little damage results from it.

Neither fungus sporulates on the chocolate spots, but only on leaves attacked 'aggressively', i.e. when the fungus brings about a general invasion of the leaf. Aggressive infection results from : (1) infection of damaged tissue; (2) a very heavy spotting of healthy leaves; (3) infected flowers falling on healthy leaves; and (4) senescence of lightly spotted leaves.

Chocolate spot caused by *B. fabae* is first noticed in the field on isolated plants which are heavily spotted compared with those around them, infection presumably having originated on leaves damaged by frost or some other means. Leaves of plants adjoining these foci of infection can become heavily spotted and succumb to aggressive infection, but this cannot account for the sudden and widespread increase in spotting that so often occurs throughout fields previously only lightly spotted.

Dead bean flowers are frequently covered with *Botrytis* spores, and these have previously been thought to play an important part in the aetiology of chocolate spot disease because they fall on to the bean leaves and cause aggressive infection resulting in a further output of spores. Collections of such flowers were made from several different fields, and sporulation tests clearly showed that in bean fields showing only a little chocolate spot the flowers were almost entirely infected by *B. cinerea*. Not until the typical spotting had considerably increased, due to heavy sporulation of *B. fabae* on the lower leaves of the plants, did dead flowers start sporulating extensively with this fungus. Flowers cannot therefore be considered to play an important role in the critical period when chocolate spot suddenly builds up to epidemic proportions.

In a field of beans which has remained for some time only lightly spotted, there is always a time when the lower leaves start to become senescent and that is when the disease is likely to increase suddenly due to the fungus becoming aggressive in the lightly spotted leaves. Any set of conditions which induce premature senescence will encourage the rapid development of chocolate spot providing climatic conditions are favourable for sporulation and infection. This would appear to account for the disease having been associated with apparently unrelated factors such as, for instance, deficiencies of either potassium or phosphorus. Well-balanced manuring and good drainage offer the best chance of bean crops withstanding serious, early attacks of chocolate spot.

J. RISHBETH. Infection of banana roots by *Fusarium oxysporum* f. *cubense*.

See *Ann. Bot., Lond.*, N.S., **19**, 1955, in the Press.

P. M. Goode. Infection of strawberry roots by zoospores of *Phytophthora fragariae*.

Observations on the encystment of zoospores of a strain of *Phytophthora fragariae* on strawberry roots showed that equal numbers of zoospores encysted on the roots of the varieties Huxley and Climax, susceptible and resistant, respectively, to the particular strain of the fungus used. Zoospores also encysted in large numbers on the roots of other plants such as species of *Ranunculus* and *Geum*. Zoospores were stimulated to encyst in large numbers at the root tip zone in all the plants examined. In addition, many zoospores were entangled in the root hair zone but few zoospores encysted elsewhere on the root. In the root hair zone zoospore germ tubes grew out in all directions but no penetration either of root hair or of epidermal cells was observed, whereas in the root tip zone germ tubes penetrated the epidermis. In the roots of the resistant variety Climax (and in the roots of unrelated plants) no further development occurred, but in the susceptible Huxley roots the fungus pursued an inter- and intracellular course in the root tip, through the cortex to the stele, where, a few millimetres from the root tip, it was confined, growing in the phloem.

Sex organs were observed 3 days after infection in the cortex of the root tip, and a day later in the stele.

J. E. Crosse. Bacteriosis of stone fruits—epiphytic microflora of cherry and its significance in leaf scar infection.

J. M. Hirst. Formation and persistence of water droplets on leaves.

Phytophthora infestans forms and disperses sporangia practically every day during a potato blight epidemic, infection is less frequent. Deposition of viable sporangia is probably greatest between 08.00 and 12.00 G.M.T. (Hirst, *Trans. Brit. mycol. Soc.* **36**, 375–393, 1953), infections may occur only when leaves remain wet long enough to allow germination and penetration. Covering healthy plants with bell jars soon after deposition of sporangia greatly increased the number of infections developing. A special recording balance was used to study the formation and persistence of water deposits on a cut potato shoot (Hirst, *Quart. J. R. met. Soc.* **80**, 227–231, 1954). Records from the balance were confirmed and explained by vertical dew-point profiles through potato crops (Hirst, Long & Penman, *Proc. Toronto Meteorological Conference, 1953*, pp. 233–237, 1954) and the time when rain fell. Records of climate do not show the variable time leaves remain wet after rain or the period when leaves are wetted by dew.

D. S. Kirkham. Chemical constituents of the host in relation to infection by *Venturia* spp.

15 October 1954. Meeting held at Birkbeck College, London, W.C. 1, at 10.45 a.m. The President, Dr S. D. Garrett, in the Chair.

Ecology of higher fungi

Miss E. M. Wakefield. Introduction.

Ecology, as applied to the fungi, is concerned with the relations of these plants to their habitats and the reasons for their distribution, whether in small areas or throughout the world. We are concerned here chiefly with the larger saprophytic species, and one of the main problems is to decide whether distribution is decided by the accompanying vegetation, or by other factors. As compared with phanerogams there are special difficulties encountered in the study of the ecology of fungi—the dependence of identification on sporophores, which are not necessarily always produced, even when weather conditions may be favourable—and the difficulties of identification of some species. Special attention was drawn to the necessity for making observations over a long period of years, and at frequent intervals during each year. As examples of ecological work some recent papers notably by Wilkins *et al.*, Jules Favre, Morten Lange and Olaf Andersson were mentioned and briefly summarized.

Valuable field notes and records could be compiled by local Natural History societies with some expert supervision, but eventually field observations must be followed by cultural and experimental work.

A. F. Parker-Rhodes. Some typical communities of Basidiomycetes.

The ecology of the higher fungi is beset with difficulties not encountered with higher plants, of which the greatest is that the species can only be identified when fruiting, which they do briefly and often very irregularly. This makes reliable sampling methods difficult to devise. The study therefore remains in a backward state.

The principal characteristics of a Basidiomycete association which can be quantitatively assessed may be listed as: proportion of ground occupied, stratification, number of niches (i.e. of species), and rate of succession. The principal edaphic factors likely to affect these characteristics are: duration and extent of the plant association, its liability to erosion, rate of access of organic matter to the humus, and chemical composition of the latter. One of the main links between the edaphic factors and the type of fungus community established is in the possibilities for the growth of ring-forming mycelia.

In Armerietum we have an extensive but short-lived (say 10 years) association of very uniform structure and rapid erosion (at least under zooplethysmic conditions). The mycota is poor in species but in old stands may attain 100% occupation; several species are specialized to the habitat (*Agaricus semotus*, *Chamaeota naucina*).

In Pteridietum the association is extensive and long-lasting, and affords a moderately high rate of access; ring-formation is slow or lacking, and the layered humus makes possible a more than 100% occupation in some areas. The mycota is rich and varied (though less than in woods), and includes both saturants (species occupying the whole available area of their niches) and subvalents (restricted to temporary vacancies between others).

In Graminetum, of the open and heavily grazed type, there is a very extensive and long-lasting association of simple soil structure and low rate of access. The fungus community is open, consisting almost wholly of ring-mycelia; the total occupation is well below 50%, and this makes possible the simultaneous presence of a large number of species of almost identical ecological requirements. The mycota is therefore rich but not very varied ecologically. There are no saturants or subvalents.

In hygrophytic consociations we find a variety of communities in close proximity, commonly due to zonation by height of water-table, etc.; the rate of access may be fairly high, and duration long. Such conditions afford a large number of niches but most are of limited extent and ring-formation is not typical; the mycota is fairly varied, and locally high rates of occupation may be attained, but there is little evidence of stratification. Many species have very sparse populations.

The observations on which this work was based were made on Skokholm Island, Pembrokeshire, Wales.

Miss L. E. Hawker. Distribution of hypogeous fungi.

As a result of a number of collections of various species of hypogeous fungi, mainly from Gloucestershire and Somerset, it was possible to arrive at some idea of the factors controlling the production of fruit-bodies of some species. The H-ion content and water-holding capacity of the soil were of primary importance. While most species were favoured by an alkaline reaction of the soil, others were most frequently found in an acid soil. Temperature and rainfall influenced the seasonal incidence of these fungi. The nature of the tree cover and of any herbaceous plants or mosses present was also important. The probable part played by animals in spore dispersal was discussed. (See *Philos. Trans.* **237**, 429–546, 1954.)

J. H. Burnett and Miss M. E. Kennedy. Experimental taxonomy of fungal populations.

The application of mating-type analysis to the study of the ecology of the higher fungi is discussed. By this method an estimate of the extent of the mycelium of an individual fungus in nature may be determined and the relationships between individuals within a population and between populations established. Such autecological studies are being made with *Polyporus betulinus* and other fungi, and the results so far obtained were discussed. Similar techniques may be used to demonstrate genetic diversity amongst closely related forms which show reduced interfertility. This is exemplified by the work being carried out on the *Nidularia denudata* and *Laccaria laccata* complexes.

J. T. Palmer. Observations on sand Gasteromycetes.

Eighteen species of epigeous Gasteromycetes were discussed, four typical sand fungi in detail. Some morphological aspects of *Geastrum nanum* and its resultant confusion with other species are outlined. The recorded habitats of *Tulostoma brumale* were discussed in the light of its alleged association in Scandinavia with *Tortula ruraliformis*, which does not seem to hold for this country.

General conclusions reached are that sand Gasteromycetes tend to be confined to particular zones of the dunes and plant associations do not seem to have the importance assigned to them by various workers. Many species of Gasteromycetes appear to be calcicoles.

T. J. Wallace. Sand dunes.

See the account of the larger fungi of Dawlish Warren. *Rep. Devon Ass. Adv. Sci.* **86**, 201–210, 1954.

J. Ramsbottom concluded the discussion with a Summary.

ANNUAL GENERAL MEETING

Saturday, 11 December 1954. The fifty-eighth Annual General Meeting was held in the rooms of the Linnean Society of London at 12.0 noon, with the President, Dr S. D. Garrett, in the Chair.

After the minutes of the previous Annual General Meeting had been read and signed, the President recorded with regret the deaths during the year of Mr A. A. Pearson, Prof. R. E. D. Baker and Dr W. Mathias. He then briefly reviewed the year's activities.

The Treasurer presented his Annual Statement, which was accepted *nem. con.*

The following Officers, Council and Committee Members were elected for 1955:

President: Dr Lilian E. Hawker.

Vice-Presidents: H. J. Howard in addition to past Presidents, C. T. Ingold and S. D. Garrett.

Secretary: J. Webster.

Foray Secretary: F. B. Hora.

Plant Pathology Committee Secretary: J. M. Hirst.

Treasurer: W. Buddin.

Editors: P. H. Gregory and G. C. Ainsworth.

New Members of Council: Miss A. H. S. Brown, D. A. Reid and J. Savory (to replace Mrs M. P. Topping, M. B. Ellis and G. Smith).

New Members of the Plant Pathology Committee: E. W. Buxton, D. J. Griffiths and D. Rudd Jones (to replace S. H. Crowdy, P. H. Gregory and E. Lester).

J. Brooke was elected to the Foray Committee.

W. Buddin was then elected an Honorary Member of the Society in recognition of his eminence as a mycologist and his great service to the Society.

Sixteen new members and two Associates were elected, making a total of forty-two new members and nine Associates for the year. A brief discussion of the programme for 1955 then followed.

In the afternoon, the President welcomed relatives and colleagues of the late Mr A. A. Pearson to hear the Pearson Memorial Lecture, entitled 'Mycology and the Amateur', given by Dr J. Ramsbottom. The meeting concluded with tributes to Mr Pearson and thanks to Dr Ramsbottom by the Vice-President, Mr W. D. Graddon.

RECEIPTS AND PAYMENTS for the year ended 30 June 1954

	£	s.	d.	£	s.	d.
1 July 1953, Balance brought forward ...				2059	4	3
Cambridge University Press Sales:						
Transactions, Vol. 36	832	6	6			
Reprints	232	16	6			
Back Volumes	155	15	10			
50-Year *Index* and *Lichen Catalogue* ...	55	5	4			
Miscellaneous (including adverts.) ...	37	0	0			
*List of Common Brit. Pl. Diseases**	4	18	9			
				1318	2	11
Sales:						
Lichen Catalogue to Brit. Ecol. Soc. ...	40	0	0			
Sundry at Meetings, etc.	35	3	0			
				75	3	0
Subscriptions:						
Arrears	6	0	0			
Current	824	19	8			
In advance	10	5	0			
Associates	24	5	6			
Income-Tax Repayment	87	9	5			
				952	19	7
Donations:						
Cheesman and Pethybridge Funds ...	2	10	0			
Plates and Printing	9	15	0			
Hospitality Fund	7	9	0			
				19	14	0
Grants:						
Through Royal Society (1953)	400	0	0			
Imp. Chem. Ind. Ltd. (2nd instalment) ...	200	0	0			
Brit. Ecol. Soc. towards printing *Lichen Catalogue*	100	0	0			
				700	0	0
Interest:						
Capital Reserve Fund	30	7	6			
Post Office Savings Bank	23	10	4			
				53	17	10
				£5179	1	7

	£	s.	d.	£	s.	d.
Wreath (A. A. Pearson)				4	0	0
Cambridge University Press:						
Production, *Transactions*, Vol. 36 (1100 copies)	1313	18	11			
Reprints	162	15	10			
Distribution of Members' copies, etc. ...	51	12	6			
Commissions on:						
Production, Vol. 36 and Reprints ...	191	0	11			
Sales of Vols. 1–36 and Miscellaneous ...	126	8	9			
Index and *Lichen Catalogue*	13	16	4			
				1859	13	3
Purchase of Reprints ...				16	18	9
Purchase of Savings Certificates				150	0	0
Printing:						
Lichen Catalogue	204	1	1			
Samp. & West., *Dis. of British Grasses* ...	384	8	2			
Sundry	50	13	11			
				639	3	2
Postages, Stationery, etc.				76	17	1
Fees for Meetings				9	7	0
Subscriptions:						
Woolhope Club	1	0	0			
British Co-ordinating Committee for Nature Conservation	2	0	0			
Council for Promoting Field Studies (2 years)	4	4	0			
				7	4	0
Hospitality Fund				10	11	0
Grants on Cheesman and Pethybridge Funds				13	0	0
Balance:						
At Midland Bank	844	12	4			
In Post Office Savings Bank	1547	15	0			
				2392	7	4
				£5179	1	7

CAPITAL RESERVE FUND (Nominal Values of Stock).

Cheesman and Pethybridge Funds ...	£260 at 3½% War Stock
Lister Fund	£300 of Savings Certificates
General, Hospitality, Library and Printing Funds	£365 of 3½% War Stock £525 of Savings Certificates £200 of 3½% Defence Bonds
GRAND TOTAL £1650	

Examined and found correct (*Signed*) W. C. MOORE; (*Signed*) WALTER BUDDIN, *Hon. Treasurer.*

THE SPRING FORAY

Lake District, 27 May to 1 June 1954

By F. B. HORA, *Foray Secretary*

The twenty-fifth Spring Foray was centred on The Ferry House, Far Sawrey, Ambleside, which is half-way down the west coast of Lake Windermere and is now the headquarters of The Freshwater Biological Association. Not only did we have full use of the excellent laboratory and library, but nearly all the thirty members who attended were able to be accommodated on the same premises. All this was made possible by the kind permission of the Director, Mr Cary Gilson, and I take this opportunity of recording our sincere thanks to him. I would like to add, too, that no praise was too high for the excellent standard of comfort and food.

Members will recall that the spring weather of 1954 was generally fine, and the Lake District was no exception. In fact, the weather during the Foray could almost be described as 'glorious'.

On Friday, 28 May, a round trip of the lakes was made taking in Rydal, Grasmere, Thirlmere, Derwentwater and returning via Ullswater. Collecting was carried out at selected areas.

On Saturday morning, Mr Cary Gilson gave a masterly talk entitled 'General Limnology', which was greatly appreciated. In the afternoon, Blelham Tarn and some nearby woods were visited in addition to some small tarns, one of which, on a former quarry site, is calcareous and has an interesting blue-green algal flora. At the same time, members took the opportunity of visiting Wray Castle, which was formerly the headquarters of the Freshwater Biological Association. Wray Castle itself is no longer inhabited but the extensive grounds are still very pleasant.

All day Sunday was spent visiting three ecologically interesting areas: Stribers Moss, Roudsea Wood (which is on limestone) and the neighbouring estuarine sands. The next morning, Dr Lund, of The Ferry House staff, gave a most instructive talk on 'Some factors affecting the growth of Phytoplankton'. In the afternoon, following a visit to 'The Hatcheries', where fish are bred for stocking and experimental purposes, the land adjoining the north end of Esthwaite was searched.

In addition to more or less terrestrial collecting, a number of members made collections from tarns and lakes as well as from baited traps which had been set some weeks previously in Lake Windermere.

A number of us took the opportunity of visiting various Departments of The Ferry House and learning something of the problems that are being tackled. We were also fortunate in seeing a demonstration—of both drawings and microphotographs—of various aspects of the Chytridiales. This demonstration had been set out by Mrs Lund, who has made, and continues to make, such outstanding contributions to this most difficult group.

A most memorable experience of this Foray was the exhibition of a selection of water-colour paintings of toadstools made by the late Miss Beatrix Potter, well known as the authoress and illustrator of many popular children's books. For myself, they are, of their kind, the best I have seen. Not a single detail has been missed. The exhibition was made possible by the kindness of Mr B. L. Thompson of The Armitt Library, Ambleside, where the paintings are normally kept and may be seen at any time.

As regards the species collected, little need be added to what the lists show. Mr Graddon was well pleased with his haul of Discomycetes whilst the numbers of species of Hymenomycetes was unusually large, no doubt mainly due to the presence of members who are interested in that group, which evidently requires much more intensive study at this time of the year than it seems to get. This applies especially to agarics.

It only remains for me to thank those who made our stay in the Lake District so agreeable. I would especially mention Dr and Mrs Lund, who not only gave me hospitality on a preliminary visit, but suggested collecting areas and arranged for me to visit all of them. They also gave us freely of their time during the actual Foray. For supplying me with species lists, it is a pleasure to thank Miss E. M. Blackwell, Dr Lilian Hawker and Miss Grace Waterhouse; Messrs W. D. Graddon and D. M. Henderson, Prof. C. T. Ingold, Mr J. T. Palmer, Dr A. F. Parker-Rhodes, Mr Derek Reid and Dr John Webster.

Lists of species recorded

Nomenclature. This follows the same lines as given in full in my Report of the Autumn Foray at Southampton, to which the reader is referred. I should add, however, that in the case of the Aphyllophorales, which is almost entirely the work of Dr Parker-Rhodes, I have added after each species in italics, the genus which Dr Parker-Rhodes himself would prefer. I may add that Dr Parker-Rhodes has agreed to my using the 'old' nomenclature for the purpose of this list.

Abbreviations. *B.* = Blelham, including the tarn; *E.* = north end of Esthwaite; *FS.* = Far Sawrey; *FS-W.* = road between Far Sawrey and Wray; *G.* = Glencoyne Wood, at head of Ullswater; *Gr.* = Grasmere; *M.* = Matterdale; *RW.* = Roudsea Wood; *Ry.* = Rydal; *SM.* = Stribers Moss; *T.* = Thirlmere; *W.* = Wray; *Wi.* = Windermere. * = species additional to the list of Discomycetes. [1] = voucher specimen in herb., Royal Botanic Garden, Edinburgh; [2] = voucher specimen in herb., University of Sheffield.

PHYCOMYCETES

Achlya colorata Pringsh., *Wi.* (lake).
Albugo lepigoni (de Bary) Kuntze, on *Spergularia marginata, SM.*
Olpidiopsis sp. *Wi.* (lake).
Peronospora arborescens (Berk.) de Bary, on *Meconopsis cambrica, Ambleside*; calotheca de Bary, on *Galium aparine, T.*; effusa (Grev.) Rabenh., on *Chenopodium album, SM.*
Plasmopara nivea (Ung.) Schroet., on *Aegopodium podagraria, Wi.*
Pythium proliferum de Bary, *Wi.* (lake); undulatum Peters, *Wi.* (lake); sp. *Wi.* (lake).
Rozellopsis septigena (Fisch.) Karl., *Wi.* (lake).
Saprolegnia ferax (Gruith) Thuret, *Wi.* (lake).
Synchytrium taraxaci de Bary & Woron., on *Taraxacum officinalis, SM., Wi.*

EU-ASCOMYCETES

PYRENOMYCETES

[2]Leptosphaeria eustoma (Fr.) Sacc., on wheat stubble, *SM.*; [2]fuckelii, on *Phalaris arundinacea, T.*; [2]typharum, on *Typha angustifolia.*

Loramyces juncicola Weston, on *Schoenoplectus lacustris*, *Wi.* (lake); macrospora Ing. & Chap., on *Juncus articulatus*, *Little Langdale Tarn.*
[2]Rabentischia typhae sensu Müller, on *Phragmites communis*, *B.*, *Wi.*
Xylaria carpophila, *FS.*

DISCOMYCETES

Aleuria lilacina Boud., *G.*; umbrina, *G.*
Anthracobia melaloma, *G.*
Apostemidium guernisaci, on twigs, *Ry.*, *Wi.*; sporogyrum Ing., on *Juncus* sp., *Little Langdale Tarn.*
Arachnopeziza aurata, *G.*
*Belonidium rhenopalaticum Rehm, on *Phragmites*, *E.*, *RW.*, *Wi.*
Belonopsis excelsior (Karst.) Rehm, on *Phragmites*, *Ry.*, *Wi.*; pullum (Phill. & Keith) Nannf., *E.*, *Wi.*
Chlorosplenium aeruginosum, *B.*
Ciliaria scutellata, *Wi.*
Colpoma quercinum, *Wi.*
*Dasyscypha albido-rosea (Rehm) Dennis, *M.*; controversa, *B.*; crystallina, *T.*, *Wi.*; diminuta, *M.*; nivea, *Wi.*; palearum (Desm.) Mass., *E.*; sydowii, *E.*; virginea, *Wi.*
Hyaloscypha stevensonii (B. & Br.) Nannf., *Wi.*
Mitrula paludosa Fr., *B.*
Mollisia arundinacea (DC.) Phill., *E.*
*Niptera poae (Fuckel) Rehm, *T.*
Ombrophila clavus, *FS.*; *var. grandis Boud., *T.*
Orbilia coccinella, *G.*; inflatula Karst., *T.*; xanthostigma, *T.*
Pachyella depressa, on decaying wood, *Ry.*
Phialea cyathoidea (Fr.) Gill., *B.*
Pyrenopeziza petiolaris (Fr.) Nannf., *T.*
Rhytisma acerinum, *Wi.*
Sclerotinia curreyana, *M.*; sulcata, *B.*
Tapesia evilescens, *E.*
Tricharia gilva, *G.*
*Trichobelonium retincolum Rehm, *B.*, *Ry.*
Vibrissia truncorum, *G.*

HETERO-BASIDIOMYCETES

TREMELLALES

Auricularia auricula-judae, on *Sambucus*, *FS.*
Calocera cornea, on *Quercus*, *FS.*, on *?Taxus*, *Ry.*
Dacrymyces stillatus Fr. nec Rea, on *Abies*, *FS.*, *T.*; succineus, on *Abies*, *FS.*, on *Pseudotsuga*, *T.*
Exidia intumescens, on *Ilex*, *T.*; spiculosa (Gray) Sommerf., *G.*; thuretiana, *T.*

UREDINALES

Cumminsiella mirabilissima, *Clappersgate.*
Melampsora tremulae (M. rostrupii), on *Mercurialis perennis*, *Coniston*, *Wi.*
Milesia polypodii, on *Polypodium vulgare*, *W.* (Castle).
Phragmidium mucronatum, *SM.*; violaceum, on *Rubus*, *Wi.*
[1]Puccinia betonicae, on *Stachys betonica*, *R.*, *W.* (Castle), *FS.*; caricina, I on *Urtica dioica*, III on *Carex*, *B.*; caricina (P. pringsheimiana), on *Ribes uva-crispa*, *FS.*; chaerophylli, on *Myrrhis odorata*, *Coniston*; chondrillae, *FS.*; epilobii, *B.*; major, *G.*; primulae, on *Primula vulgaris*, *W.* (Castle); [1]sessilis (P. phalaridis), *Ry.*, on *Arum maculatum*, *Ry.*, *SM.*, *Wi.*; sessilis (P. winteriana), on *Allium ursinum*, *Ferry House*, *Wi.*; tumida, on *Conopodium majus*, *G.*; violae, on *Viola* sp., *Wi.*
Trachyspora intrusa, on *Alchemilla vulgaris*, *T.*, *W.* (Castle).
Tranzschelia anemones, on *Anemone nemorosa*, *Keswick*, *W.* (Castle).
Triphragmium ulmariae, *B.*, on *Filipendula ulmaria*, *G.*, *Wi.*

Uromyces dactylidis, on *Ranunculus ficaria*, *FS.*, *G.*; ficariae, on *R. ficaria*, *G.*, *T.*, *W.* (Castle); scillarum, *FS.*; valerianae, *B.*

Xenodochus carbonarius, *B.*

USTILAGINALES

Entyloma ficariae, *Rosthwaite, Borrowdale.*

Urocystis anemones, *Derwentwater, FS.*

Ustilago striiformis, on *Holcus mollis*, *B.*

HOMO-BASIDIOMYCETES—HYMENOMYCETES

APHYLLOPHORALES

Corticium pruinatum Bres. (*Botryobasidium*), on *Abies*, *FS.*; roseum (*Aleurodiscus*), *T.*; sambuci, on *Sambucus*, *FS.* (lakeside), *SM.*; subcoronatum (*Botryobasidium*), on burnt rotten *Quercus*, *SM.*

Cyphella villosa, *Ambleside.*

Daedalea confragosa, on *Salix*, *E.*; quercina, on *Quercus*, *G.*

Fomes annosus, on *Abies*, *T.*; ferruginosus (*Physisporus*), on *Quercus*, *FS.*; igniarius var. nigricans (*Phellinus*), on *Betula*, *G.*

Gloeocystidium leucoxanthum Bres. (*Vuilleminia*), on *Quercus*, *FS-W.*

Hymenochaete rubiginosa, *RW.*

Hypochnus botryoides (*Prilleuxia*), on *Abies*, *FS.*

Lenzites betulina, *W.*; sepiaria (*Gloeophyllum*), *Tarn Hows.*

Odontia bicolor, *T.*; crustosa, on *Pseudotsuga*, *FS.*, *T.*; papillosa, *T.*

Peniophora accedens, on *Salix*, *E.*; ? aegerita, on *Salix*, *E.*; cinerea (*Gloeopeniophora*), on *Quercus*, *G.*; laevis, on *Betula*, *SM.*; molleriana, in moss on *Quercus*, *G.*; pallidula, on *Larix*, *T.*; pubera, *RW.*

Polyporus brumalis (*Leucoporus*), *B.* on *Quercus*, *G.*, *FS.* on *Corylus* and *Alnus*, *SM.*

Polystictus versicolor (*Coriolus*), on *Quercus*, *G.* on *Corylus*, *RW.*

Poria medulla-panis, on *Salix*, *E.*; sanguinolenta (*Podoporia*), on *Salix*, *E.*; versipora (Pers.) Baxter (*Xylodon*) (=Poria mucida, vaporaria sensu auct. angl.), on *Salix*, *E.*; on *Fagus*, *FS.*; viridans (*Tyromyces*), on *Betula*, *SM.*

Stereum hirsutum, on *Quercus*, *G.* on *Fagus* and *Quercus*, *FS.* (lakeside) on *Quercus*, *S.* var. luteocitrinum, on *Fagus*, *FS.*; rugosum, on *Salix*, *E.*, *FS.* on *Quercus*, *G.* on *Betula*, *RW.*, *T.*; spadiceum, *T.*

Trametes mollis (*Antrodia*), on *Fagus*, *FS-W.*; serialis (=Poria callosa (Fr.) Cke.), on *Betula*, *SM.*

AGARICALES

Collybia palustris, *M.*, ?*SM.*

Coprinus boudieri, *B.*; plicatilis, *B.*, *FS.*; pyrranthes Romagn., *G.*

Cortinarius sp., *M.*

Galera clavata Vel., *E.*; hypnorum, *G.*, *SM.*, *T.*; mniophila, *B.* (Red Pike).

Hypholoma fasciculare, *RW.*, *T.*

Lactarius ? torminosus, *E.*

Marasmius dryophilus, *B.*, *FS.*, *SM.*

Mycena acicula, *B.*; alcalina, *E.*, *T.*; amicta, *B.*; galericulata, *B.*, *FS-W.*, *RW.*; quisquiliaris, *M.*

Omphalia ? gibba, *E.*, *M.*; integrella, *E.*; umbellifera, *M.*, *G.*, *SM.*; var. nivea, *B.* (Red Pike).

Panaeolus campanulatus auct., *B.*, *E.*, *FS.*; var. sphinctrinus, *B.*

Pholiota mutabilis, *RW.*; mycenoides, *E.*

Pluteus cervinus, *FS.*; chrysophaeus, *RW.*

Psathyrella pennata, *G.*

Russula cyanoxantha, *FS.*

Stropharia semi-globata, *B.*; melanosperma, *RW.*

Tricholoma gambosum, *Ferry House grounds.*

Tubaria furfuracea, *G.*; ? gibbosa Favre, *B.* (Red Pike); minutalis Romagn., *FS-W.*

GASTEROMYCETES

Cyathus striatus, *RW.*
Phallus impudicus, *G., Nab Wood, RW.*

FUNGI HYPOGEI

PHYCOMYCETES

Endogone lactiflua, *T.*

ASCOMYCETES

Elaphomyces granulatus, *T.*

FUNGI IMPERFECTI

COELOMYCETES

Actinothyrium graminis, *B.*
Ceuthospora phacioides, *FS.*
Dinemasporium graminum, *M.*, on *Agropyron repens* and *Juncus maritimus*, *SM.*, on *Phragmites communis*, *Wi.*
Heteropatella bonordenii, *B.*

HYPHOMYCETES

Alatospora acuminata Ing., on decaying alder and oak leaves, *Gr.*
Articulospora tetracladia Ing., on decaying alder and oak leaves, *Gr.*
Clavariopsis aquatica de Wild., on decaying alder and oak leaves, *Gr.*
Oidium sp., on *Filipendula ulmaria*, *G.*
Ovularia bistortae, *B.*
Periconia atra, *M.*
Tetracladium marchalianum, on decaying alder and oak leaves, *Gr.*; setigerum (Grove) Ing., on decaying alder and oak leaves, *Gr.*
Varicosporium elodeae Kegel, on decaying alder and oak leaves, *Gr.*

THE NEW FOREST FORAY

7 to 14 September 1954

By F. B. HORA, *Foray Secretary*

The fiftieth Autumn Foray of the Society was centred on the University of Southampton by kind permission of the Vice-Chancellor, Mr D. G. James, who welcomed us at a reception after dinner on Tuesday, 7 September. About 70 members attended and were accommodated at the Glen Eyre Hall of Residence, where the Warden, Major Mead, and Vice-Warden, Dr Wilson, went out of their way to ensure that we were all well housed and well fed. For the examination of specimens, Prof. Williams gave us full and free use of his Department of Botany. To all these gentlemen the Society is greatly indebted. They will be in no doubt as to the sincere thanks of those members who benefited so much from their efforts.

The Society has visited the New Forest area for Autumn Forays on two previous occasions, in 1899 and 1916. It is always tempting to make comparisons and this can be done, in a very broad way, in terms of the number of agarics collected, since this is the group most consistently gathered on these occasions. Complete figures are not available for 1899, since only the 'more uncommon' species were listed in our *Transactions*, but the complete list for 1916 showed some 325 agarics. This was an exceptionally good haul. (From published lists, especially of the late J. C. Rayner, who was a member of our Society, I estimate the *recorded* agaric flora of the New Forest area to be not less than 500 species.) The Dunkeld Foray of 1953 yielded some 300 species and we considered that to be very good. I remember the late A. A. Pearson telling me that the most prolific week's foray he attended (it was in France in company with the late Dr Paul Konrad) yielded about 400 agarics. I should estimate an 'average' autumn foray to yield about 250 agarics. Our present foray list shows just over 200 species, so that on the above very rough computation it was below average. It must be remembered that it is usually easy to collect the first 50 species, but there must be much more 'abundance' to collect *another* 50 species when one has already got the first 200. These rough calculations do undoubtedly give some sort of numerical basis to what was the general impression of members who attended the foray, namely, that the season was not a 'good' one. The reason for this is most probably to be sought in the exceptionally bad weather of the summer months. A really fine spring, up to and including Whitsuntide, was followed by depressing and almost continuous rain—downpours at times—as well as relatively low temperatures. At a London meeting on 15 October 1954, Dr Lilian Hawker stressed the importance of excessive rainfall, lack of aeration and low temperatures in hindering the maturation of fruit-bodies of hypogeous fungi. I have no doubt that similar conditions affect the production of subaerial fruiting bodies of toadstools in much the same way. One must always bear in mind that the main 'body' of a toadstool, at least in terrestrial species, develops underground, as a mycelium. There is good evidence to indicate that the mycelium must reach a certain stage of development before fruit-body production can occur.

The first collecting area to be visited was on 8 September when an area of mixed deciduous woods and conifer plantations around Ashurst was explored. Collecting was done under almost continuous rain or drizzle. One might, on first principles, expect the 'hygrophanous state' of toadstools to be seen at its best, but even that was often 'water-logged' beyond easy recognition. Nevertheless, some interesting species were collected: the rare *Plasmopara epilobii*, the uncommon *Pseudoperonospora urticae* and that striking and by no means common species *Stereum multizonatum*. Fine specimens of *Pleurotus lignatilis* caused some puzzled expressions on account of the virtually central stems. The afternoon was spent examining the specimens. On the same evening, after dinner, the President, Dr S. D. Garrett gave his presidential address: 'Microbial Ecology of the Soil'. It was very warmly received and a hearty vote of thanks was given by the Vice-President, Mr W. D. Graddon.

After a very stormy and boisterously windy night with no signs of abatement throughout breakfast the following morning (Thursday), the prospects were anything but promising for a whole day at Studland Bay to examine a sand-dune flora. But the weather suddenly cleared from about 10 a.m. onwards, and a sunny, invigorating and bracing day was spent on this most interesting ecological area. It is evident that intensive investigation of the fungus flora of such areas throughout the country would be very rewarding. Fine specimens of *Corynetes arenarius* were seen and a form of *Puccinia sonchi* with unicellular teleutospores was found. According to Dr O'Connor, only some half a dozen collections have been made of this species, and about half of them have unicellular teleutospores. I note that Grove described this rust as 'widely different from a typical *Puccinia*'. On the usual arrangement, a one-celled teleutospore would place a rust in the genus *Uromyces*. It is interesting to note that nearly forty years ago, when reviewing the literature on certain aspects of rusts, Dr Ramsbottom noted that at that time the opinion was being expressed that too much emphasis was being placed on the teleutospore as a classificatory unit. Another good find was the rare *Puccinia hydrocotyles*, and Mr Peter Austwick reported the largest specimens of *Polystictus perennis* he had ever seen. Of agarics, numerous specimens of the almost annulate *Inocybe carpta* sensu Heim were taken. A fine day's collecting was rounded off by an excellent tea, whilst the long trek back to the ferry was eased for many of us by Mr John Brooke running a high-speed shuttle service with a large car.

On Friday morning, the Studland Bay material was worked through and the same afternoon, a damp oak-wood at Rufus Stone was visited. Damp is scarcely the word: the ground was sodden and a periodic drizzle accompanied most of the collecting. Just before setting off on the return journey, a torrential downpour occurred, lasting about a quarter of an hour, and many members were caught some distance from the bus. Notable finds in this area were: *Thekopsora vaccinorum*, *Neobulgaria pura*—

the latter appearing to be quite widely distributed in the country though not yet in our list—*Amanita virosa*, *Cortinarius subtortus*, *Marasmius hudsoni* and *Pluteus villosus*.

It was, most unfortunately, found necessary to alter the advertised programme and make Saturday the Free Day instead of Sunday. The rain held off and it was a bright though windy day. A number of small parties visited the Isle of Wight, and one or two members attended the Farnborough Air Display. Others took the opportunity for further collecting or working over material already collected. In the evening, the Society gave a dinner in honour of the Vice-Chancellor. The President presided, introducing Mr James and thanking him in a most witty speech. The Vice-Chancellor's equally effective reply was followed by Dr O'Connor who paid tribute to all others who had done so much for the success of the Foray.

On Sunday afternoon, the Denny area was visited under quite pleasant conditions. *Darluca filum* was taken, the perfect stage of which has been reported from the tropics and is known as *Eudarluca filum* Speg. (Sphaeriales). There was also *Cercospora lythri*, the only previous record of which appears to be from Ireland in 1938. *Polyporus albellus* was collected and Mr Peter Austwick tells me this is probably the first time this species has been recognized on a Foray, for it is usually confused with *P. lacteus*. He also identified *Solenia candida*, almost certainly widely distributed, but overlooked on account of its small size. It was a pleasure to gather what subsequently turned out to be the rare *Mycena floccipes*. It is in the British list but was never seen by Carleton Rea, at least up to the time of the preparation of his flora.

The morning of the last collecting day, Monday, 13 September, was spent in the Knightwood area (including Vinney Ridge). The day was fine and two new records were added to our lists: *Rhizopogon roseolus* and *Tubaria pallidispora*. The presence of larch canker, the causal organism of which has for years been known under the name of *Dasycypha calycina*, recalls that Dr Manners has recently shown (these *Transactions*, **36**, 362 (1953)) that this name should give place to *Trichoscyphella hahniana*. There was also the rare *Puccinia veronicae*. The various collections were worked over in the afternoon, and on the following day, the last of our members departed.

In retrospect, there can be no doubt that the Foray was highly satisfactory. Any lack of fungal abundance was more than compensated by the interest of many of the finds. At all available times, the Botanical laboratories were, if anything, always overcrowded with keen workers. Never, since attending Forays, have I seen so many microscopes in active use, especially by so many *young* people. This should warm the hearts of the authors of a Report entitled 'The need for encouraging the study of Systematic Mycology in England and Wales' (these *Transactions*, **32**, 104 (1949)). *Post hoc ergo propter hoc!?*

The large increase in the number of members who attend an Autumn Foray, makes the organization, running and reporting of such Forays more than ever dependent on the friendly co-operation of numerous people. I have always been particularly fortunate in this respect. I have already mentioned a few by name and now offer them my sincere thanks. It is also a pleasure to include Dr Agnes Brown, Dr Lilian Hawker, Miss Margaret Holden, Miss Grace Waterhouse, Messrs John Brooke, W. D. Graddon, H. J. Howard, Dr John Manners, Messrs P. D. Orton, J. T. Palmer, Michael Parker and T. J. Wallace. I would like especially to thank Dr Manners who acted as local secretary. The smooth running of the Foray is a tribute to his masterly powers of organization.

Lists of species recorded

Introduction. In view of the increasing interest in the hypogeous fungi, I have, for convenience of reference, separated out the species of this group under the heading 'Fungi hypogei'.

Nomenclature. Where author-citation is given, I have been guided, where necessary, by specialists in the groups concerned. Where there is no author-citation, I have used the binomial to be found in the appropriate one of the following publications which are arranged in alphabetical order of the main groups.

Angiosperms (as host plants), A. R. Clapham, T. G. Tutin & E. F. Warburg, *Flora of the British Isles* (1952); Cambridge University Press.

Ascomycetes. Discomycetes: List of Discomycetes recorded for the British Isles, by J. Ramsbottom & F. L. Balfour-Browne; these *Transactions* **34**, 38 (1951). Pyrenomycetes: List of Pyrenomycetes recorded for Britain, by G R. Bisby & E. W. Mason; these *Transactions*, **24**, 127 (1940).

Basidiomycetes. Agaricales: Revised List of British Agarics and Boleti, by A. A. Pearson & R. W. G. Dennis; these *Transactions*, **31**, 145 (1948), supplemented by: New Records and Observation IV, by A. A. Pearson; these *Transactions* **32**, 258 (1949); *idem*, V; these *Transactions*, **35**, 97 (1952). Aphyllophorales and Gasteromycetes: Carleton Rea, *British Basidiomycetae* (1922); Cambridge University Press. Uredinales: List of British Uredinales, by Malcolm Wilson and G. R. Bisby; these *Transactions*, **37**, 61 (1954). Ustilaginales: G. C. Ainsworth & Kathleen Sampson, *The British Smut Fungi* (1950); Commonwealth Mycological Institute.

Fungi hypogei. British Hypogeous Fungi, by Lilian E. Hawker; *Philos. Trans.* **237**, 429 (1954).

Fungi Imperfecti. W. B. Grove, Coelomycetes: *British Stem and Leaf Fungi*; Cambridge University Press (1935, 1937). Hyphomycetes: List of Hyphomycetes recorded for Britain, by E. M. Wakefield & G. R. Bisby; these *Transactions*, **25**, 49 (1941).

Myxomycetes. A. & G. Lister, *A Monograph of the Mycetozoa* (1925); British Museum (Natural History).

Abbreviations. (1) Localities: *A.* = Ashurst; *B.* = Bramshaw (visited by some members on the free day); *D.* = Denny area; *G.* = grounds of Glen Eyre Hall of Residence, University of Southampton; *K.* = Knightwood and Vinney Ridge; *R.* = Rufus Stone; *S.* = Studland Bay. (2) Others: * = species additional to above publications; † = new British record. (3) Voucher material: ^{1} = in herb. P. K. C. Austwick; ^{2} = in herb. Kew.; ^{3} = in herb. J. T. Palmer; ^{4} = in herb. University of Sheffield; ^{5} = in herb. University College, Exeter.

MYXOMYCETES

Ceratiomyxa fruticulosa, *K.*, *R.*, *S.*
Comatrichia typhoides, *D.*
Cribraria argillacea, *A.*; vulgaris, *A.*
Dictydium cancellatum var. fuscum, *A.*
Diderma floriforme, *A.*
Fuligo septica, *A.*; var. candida, *A.*
Hemitrichia vesparium, *D.*
Physarum nutans, *D.*; virescens, *K.*; var. nitens, *R.*
Reticularia lycoperdon, *D.*

Stemonitis ferruginea, *A.*; fusca, *A.*
Trichia affinis, *R.*; decipiens, *R.*; persimilis, *D.*; scabra.
Tubifera ferruginosa, *D.*, *K.*, *R.*

PHYCOMYCETES

Peronospora trifoliorum de Bary (P. lotorum Sydow), on *Lotus uliginosus*, *A.*
Plasmopara epilobii (Rabenh.) Schroet., on *Epilobium parviflorum*, *A.*
Pseudoperonospora urticae (Lib. ex Berk.) Salm. & Ware, on *Urtica dioica*, *A.*
Synchytrium succisae de Bary & Woron., on *Succisa pratensis*

HEMI-ASCOMYCETES

Protomyces macrosporus Unger, on *Apium nodiflorum*, *A.*

EU-ASCOMYCETES

Pyrenomycetes

Calosphaeria wahlenbergii, *D.*
Chaetosphaeria innumera, on *Fagus*, *D.*
Diatrype stigma, on *Betula*, *R.*
Eutypa lata, on *Fagus*, *D.*
Gloniopsis levantica Rehm, on *Rhododendron* sp.
Hypomyces aurantius, on old polypore, *A.*
Hysterium angustatum, on rotten wood, *D.*
[4]Leptosphaeria littoralis, on *Thymus arenaria*, *S.*; [4]michotii, on *Typha latifolia*, *D.*; [4]microscopica, on *Deschampsia caespitosa*, *R.*
Melanomma subdispersum (Karst.) Berl. & Vogl. (conidial), on *Fagus*, *D.*
Nectria punicea, on *Ilex*, *R.*
[4]Pleospora vagans, on *Festuca pratensis*, *A.*, on *Deschampsia caespitosa*, *R.*
Valsa ceratophora, on *Fagus*, *A.*
Xylaria hypoxylon, on various hosts, *D.*

Discomycetes

Aleuria micropus, *D.*
Anthracobia maurilabra, *R.*
Arachnopeziza aurelia, *B.*
Ascobolus denudatus, *B.*
Belonopsis excelsior (Karst.) Rehm, *S.*
Bulgariella pulla, *D.*
Calycella lenticularis, *D.*; *sulphurina (Quél.) Boud., *S.*
Chlorosplenium aeruginosum, *D.*
Ciliaria hinnulea, *B.*; scutellata, *R.*
Coprobia granulata, *A.*
[5]Corynetes arenarius (Rostr.) Dur., *S.*
Dasyscypha apala (B. & Br.) Dennis, *G.*; brevipila, *D.*; ciliaris, *R.*; crucifera, *S.*; pulchella, *B.*; pulveracea (A. & S.) v. Höhn., *S.*; virginea, *R.*
Galactinia badia, *B.*; *limosa (Grel.) Le Gal & Romagn., *A.*
Helotium fructigenum, *G.*
Helvella crispa, *D.*
Hyaloscypha leuconica (Cke.) Nannf., *D.*
Leotia lubrica, *R.*
Macropodia macropus, *R.*
*Neobulgaria pura (Fr.) Petrak, *R.*
Phaeobulgaria inquinans (Fr.) Nannf., *R.*
Propolis faginea, *A.*
Pyronema omphalodes, *A.*
Rutstroemia echinophila (Fr.) v. Höhn., *D.*; sydowiana (Rehm) White, *G.*
Stegia ilicis, on *Ilex* leaves, *R.*
Trichoscyphella hahniana (Seaver) Manners (Dasyscypha calycina auct. mult. plur.), *K.*

HETERO-BASIDIOMYCETES

Tremellales

Calocera cornea, *A.*, *D.*, *R.*; viscosa, *K.*

Uredinales

Coleosporium melampyri, on *Melampyrum pratense*, *R.*; tussilaginis, on *Melampyrum* sp., *R.*

Kuehneola uredinis, on *Rubus fruticosus*, *D.*, *K.*, *S.*

Melampsora hypericorum, on *Hypericum humifusum*, *K.*

Melampsoridium betulinum, on *Betula pubescens*, *A.*, *R.*, on *B. verrucosa*, *D.*, *K.*

Milesia blechni, on *Blechnum spicant*, *A.*, *K.*

Phragmidium rubi, on *Rubus fruticosus*, *S.*; violaceum, on *Rubus fruticosus*, *Fawley*, *K.*

Puccinia annularis, on *Teucrium scorodonia*, *A.*, *K.*; asteris, on *Aster tripolium*, *Totton salt-marsh*; cirsii, on *Cirsium palustre*, *A.*; cnici, on *Cirsium vulgare*, *Fawley*, *K.*; cnici-oleracei, on *Cirsium palustre*, *Fawley*; coronata, on *Holcus lanatus*, *A.*, *D.*, *F.*, *K.*; hydrocotyles, on *Hydrocotyle vulgaris*, *S.*; lapsanae, on *Lapsana communis*, *G.*; lemonnieriana, on *Cirsium palustre*, *R.*; menthae, on *Mentha aquatica*, *D.*, *K.*, *R.*, on *M.* × *verticillata*, *K.*; obtegens, on *Cirsium arvense*, *G.*, *K.*; poarum, on *Tussilago farfara*, *A.*; saniculae, on *Sanicula europea*, *R.*; sonchi, on *Sonchus arvensis*, *S.*; veronicae, on *Veronica montana*, *K.*; violae, on *Viola riviniana*, *A.*, *D.*, *R.*

Pucciniastrum epilobii, on *Chamaenerion angustifolium*, *G.*

Thekopsora vacciniorum, on *Vaccinium myrtillus*, *A.*, *D.*, *R.*

Uromyces limonii, on *Limonium vulgare*, *S.*; rumicis, on *Rumex hydrolapathum*, *S.*; trifolii, on *Trifolium pratense*, *G.*

Ustilaginales

Sphacelotheca hydropiperis, on *Polygonum hydropiper*, *A.*, *D.*

Urocystis anemones, on *Ranunculus* sp., *A.*

HOMO-BASIDIOMYCETES—Hymenomycetes

Aphyllophorales

Clavaria cristata, *S.*, dissipabilis, *A.*; fusiformis, *R.*; stricta, *R.*

[1]Corticium atro-virens, *D.*; sulphureum, *A.*

Daedalea biennis, *A.*, quercina, *A.*, *D.*, *R.*

Fistulina hepatica, *A.*

Fomes annosus, *A.*; ferruginosus, *A.*, *D.*

Ganoderma applanatum, *D.*

[1]Gloeocystidium roseo-cremeum (Bres.) Brinkm. (=Corticium roseo-cremeum), *R.*

Hydnum auriscalpium, *S.*; repandum, *A.*, *D.*, *R.*

Hymenochaete rubiginosa, *D.*

Merulius tremellosus, *A.*

Peniophora cremea, *A.*; gigantea, *K.*

Phlebia merismoides, *D.*

[1]Polyporus adustus, *D.*; [2]albellus, *D.*; amorphus, *A.*, *R.*; betulinus, *A.*, *D.*, *R.*, *S.*; [1]caesius, *A.*, *D.*, *K.*, *R.*, *S.*; cuticularis, *A.*; [1]dryadeus, *A.*; [1]fragilis, *A.*, *D.*, *S.*; giganteus, *A.*; [1]lacteus, *D.*; [1]rutilans, *A.*; schweinitzii, *A.*, *K.*, *D.*; [1]semi-pileatus Peck (=P. chioneus Bres. non Fr.), *D.*, *R.*; [1]stipticus, *A.*

Polystictus abietinus, *K.*; [1]perennis, *S.*

[1]Poria candidissima (Schw.) Sacc. (=P. hymenocystis), *A.*, *D.*; [1]gilvescens, *D.*; [1]versipora, *A.*, *D.*, *R.*

[2]Solenia candida, *S.*

Stereum gausapatum Fr. (=S. spadiceum Fr. non Pers.), *A.*, *D.*; hirsutum, *A.*, *D.*, *R.*; [2]multizonatum, *A.*; purpureum, *A.*, *D.*; sanguinolentum, *K.*

Thelephora terrestris, *A.*

[1]Tomentella ferruginea Pers. (=Hypochnus ferrugineus), *A.*, *D.*; [1]fusca (Pers.) Schroet. (=H. fuscus), *D.*, *S.*

Trametes gibbosa, *A.*, *D.*; [1]mollis, *D.*; rubescens, *A.*, *D.*, *S.*

AGARICALES

Amanita citrina, *A.*, *K.*, var. alba, *A.*, *K.*; excelsa, *A.*, *D.*; muscaria, *A.*, *R.*, *S.*; pantherina, *A.*; phalloides, *A.*; porphyria, *R.*; rubescens, *A.*, *D.*, *K.*, *R.*, *S.*; virosa, *R.*

Amanitopsis fulva, *A.*, *K.*, *S.*

Anellaria semi-ovata, *A.*, *D.*

Armillaria mellea, *A.*, *D.*, *K.*; mucida, *D.*, *R.*

Bolbitius vitellinus, *S.*

Boletus badius, *A.*, *R.*; bovinus, *A.*, *K.*, *S.*; chrysenteron, *A.*; edulis, *D.*; elegans, *K.*; erythropus, *K.*; felleus, *D.*; granulatus, *A.*; luteus, *R.*; scaber, *K.*, *S.*; versipellis, *A.*, *R.*

Cantharellus cibarius, *D.*, *R.*; tubaeformis, *D.*, *R.*

Clitocybe aurantiaca, *S.*; infundibuliformis, *A.*, *D.*; rivulosa, *A.*; tabescens, *A.*

Clitopilus prunulus, *A.*

Collybia cirrhata, *R.*; distorta, *D.*; fusipes, *D.*; maculata, *S.*; myosura, *S.*; platyphylla, *A.*, *D.*; radicata, *A.*, *D.*

Coprinus comatus, *D.*

Cortinarius albo-violaceus, *D.*, *K.*; anomalus, *A.*; armillatus, *D.*, *K.*; bolaris, *D.*, *K.*, *R.*; cinnamomeus, *D.*; delibutus, *R.*; elatior, *A.*, *K.*, *R.*; [5]flexipes, *S.*; [5]hemitrichus, *D.*, *R.*, *S.*; hoeftii, *A.*, *D.*; nemorensis, *A.*; obtusus, *A.*; purpurascens, *A.*; rigidus, *D.*; sanguineus, *K.*; [5]saniosus, *S.*; semi-sanguineus, *K.*; *subtortus Fr., *R.*; torvus, *D.*, *R.*; [5]uliginosus, *S.*; vibratilis, *A.*

Craterellus cornucopioides, *D.*, *R.*

Crepidotus calolepis, *S.*; mollis, *S.*; variabilis, sensu stricto, *D.*

*Eccilia carneo-alba (With.) Ricken, *S.*

Entoloma lividum, *A.*; nidorosum, *D.*, *K.*

[5]*Flammula fulgens (Favre & Maire) Kühner, *S.*; penetrans, *A.*, *K.*

Galera hypnorum, *D.*; [5]mniophila, *S.*; rubiginosa, *D.*; tenera, *D.*

Gomphidius rutilus, *A.*

Gyroporus castaneus, *D.*

Hebeloma anthracophilum, *A.*; [5]crustuliniforme, *R.*, *S.*; mesophaeum, *S.*

Hygrophorus ceraceus, *S.*; conicus, *A.*, *S.*; eburneus, *A.*; pratensis, *S.*; turundus, *S.*

Hypholoma fasciculare, *A.*, *D.*, *K.*, *R.*; hydrophilum, *D.*

[5]*Inocybe carpta sensu Heim, *S.*; [5]dulcamara, *S.*; geophylla, *A.*, *K.*; [5]halophila, *S.*; *hirtella Bres., *D.*; lanuginosa, *A.*; [5]longicystis, *S.*; maculata, *A.*; napipes, *A.*, *D.*; petiginosa, *R.*; [5]trechispora, *S.*; umbrina, *K.*

Laccaria amethystina, *A.*, *D.*, *K.*, *R.*, *S.*; laccata, *A.*, *D.*, *K.*, *R.*, *S.*; proxima, *S.*

Lactarius blennius, *A.*, *D.*, *K.*, *R.*; camphoratus, *A.*, *K.*, *R.*; chrysorrheus, *A.*, *D.*, *K.*; deliciosus, *D.*, *K.*, *S.*; glyciosmus, *S.*; hepaticus, *A.*, *K.*, *S.*

[5]Lactarius lacunarum (Romagn.) Lange, *S.*; picinus, *D.*; piperatus, *R.*; plumbeus, *S.*; quietus, *A.*; [5]rufus, *A.*, *D.*, *S.*; subdulcis, *K.*; [5]*tabidus sensu Kühner & Romagn., *A.*, *K.*, *R.*, *S.*; torminosus, *S.*; vietus, *S.*

Lepiota amianthina, *D.*

*Leptonia euchlora (Lasch) Fr., *S.*

Marasmius androsaceus, *A.*, *K.*; candidus sensu Lange, *A.*; confluens, *S.*; dryophilus, *D.*; fusco-purpureus, *D.*; hudsoni, *R.*; oreades, *S.*; peronatus, *A.*, *D.*, *K.*, *R.*, *S.*; ramealis, *D.*; rotula, *D.*

Mycena alcalina sensu Schroet., *R.*, *S.*; ammoniaca; capillaripes sensu Smith (=M. rubro-marginata sensu Pearson), *K.*; corticola; floccipes (Fr.) Kühner (=Collybia floccipes), *D.*; galericulata, *D.*; galopus, *A.*, *D.*, *K.*, *R.*, *S.*; var. alba, *D.*; haematopus, *A.*, *D.*; rorida, *A.*; sanguinolenta, *A.*, *K.*, *R.*, *S.*; stylobates, *K.*; vitilis, *D.*

Naucoria bohemica, *S.*; centunculus, *D.*; escharoides, *D.*; [5]semi-orbicularis, *S.*; muricata (Pholiota), *K.*

Nolanea staurospora, *A.*, *D.*, *S.*

Nyctalis parasitica, *A.*

Omphalia fibula, *A.*, *S.*; integrella, *S.*; umbellifera, *D.*

Panaeolus campanulatus auct., *A.*
Panus stipticus, *R.*
Paxillus atro-tomentosus, *A.*; involutus, *A.*, *D.*; panuoides, *A.*, *D.*
Pholiota mutabilis, *D.*; spectabilis, *S.*; unicolor sensu Lange, *D.*
Pleurotus ?applicatus, *S.*; lignatilis, *A.*; ostreatus, *D.*; reniformis, *D.*
Pluteus cervinus, *A.*, *D.*, *K.*; *villosus sensu Decary, *R.*
Psalliota augusta, *D.*; meleagris, *S.*; sanguinaria, *A.*; silvicola, *R.*
Psathyrella disseminata, *A.*
[5]Psilocybe ammophila, *S.*; [5]elongata, *S.*; ericaea, *A.*; foenesecii; semi-lanceata, *A.*
Russula adusta, *A.*, *R.*; alutacea, *D.*; amoena, *D.*; atro-purpurea, *A.*, *R* ; caerulea, *S.*; cyanoxantha, *A.*, *D.*, *R.*; drimeia, *A.*, *K.*, *S.*; emetica, *A.*, *D.*, *K.*, *R.*; exalbicans, *D.*; fallax, *A.*; farinipes, *A.*; fellea, *A.*, *D.*, *R.*; fragilis, *A.*, *K.*; heterophylla, *A.*; lutea, *A.*; nigricans, *A.*, *K.*; ochroleuca, *A.*, *D.*, *K.*, *R.*, *S.*; queletii, *D.*; rosea, *D.*; sanguinea, *A.*, *K.*; vesca, *A.*, *D.*, *K.*, *R.*; virescens, *A.*; xerampelina, *S.*
Schizophyllum commune.
Stropharia semi-globata, *A.*, *D.*, *R.*
Tricholoma aggregatum, *K.*; fulvum, *A.*, *K.*; loricatum, *A.*; rutilans, *A.*, *D.*, *K.*; sulphureum, *A.*, *D.*, *K.*; terreum, *A.*, *D.*; ustale, *D.*
†Tubaria pallidispora Lange, *K.*

HOMO-BASIDIOMYCETES—Gasteromycetes

Bovista plumbea, *S.*
Lycoperdon depressum, *A.*, *D.*, *S.*; ericetorum Pers., *D.*; molle, *D.*; perlatum, *A.*, *R.*; var. nigrescens, *A.*, *D.*; pyriforme, *K.*
Mutinus caninus, *A.*, *D.*, *K.*, *R.*
[3]Nidularia pisiformis, *A.*
Phallus impudicus, *A.*, *D.*, *K.*
Scleroderma aurantium, *A.*, *D.*, *K.*, *R.*; [3]bovista, *S.*
Sphaerobolus stellatus, *A.*, *R.*

FUNGI HYPOGEI

Phycomycetes

Endogone lactiflua, *A.*

Ascomycetes

Elaphomyces granulatus, *A.*, *K.*; muricatus, *D.*, *R.*
Genea hispidula, *K.*

Basidiomycetes

†Rhizopogon roseolus (Fr.) Hollós, *K.*

FUNGI IMPERFECTI

Coelomycetes

Camarographium metableticum, on *Agropyron junceum*, *S.*
Darluca filum, on Puccinia menthae on *Mentha aquatica*, *D.*; on Uromyces rumicis, *A.*
Dinemasporium graminum, on *Molinia caerulea*, *K.*
Stagonospora trimera, on *Phragmites communis*, *S.*

Hyphomycetes

Acladium conspersum Link, on *Fagus*, *D.*
Botrytis argillacea, on *Fagus*, *D.*
Brachysporium apicale, on *Fagus*, *D.*, *R.*; bloxami, on *Fagus*, *D.*, *R.*; britannicum Hughes, on rotten wood, *D.*
Cercospora lythri, on *Lythrum salicaria*, *D.*
[5]Cristulariella depraedens, on *Acer*, D.
Dactylella mammillata Dixon, on rotten wood, *D.*
Haplaria grisea, on *Fagus*, *D.*

Oidium aureum Fr., on rotten wood, *D.*, *R.*

Ovularia destructiva, on *Myrica gale*, *S.*; sphaeroidea, on *Lotus uliginosus*, *A.*; on *Lotus* sp., *G.*

Paecilomyces elegans (Corda) Mason & Hughes, on rotten bark, *D.*

Papularia arundinis, on *Phragmites communis*, *S.*

Pycnostysanus azaleae (Peck) Mason, on *Rhododendron ponticum*, *K.*

Ramularia scrophulariae, on *Scrophularia* sp.

Sepedonium sp., on *Rhizopogon roseolus*, *K.*

Stilbum tomentosum Fr. var. ovalisporum A. L. Smith, on *Trichia*, *D.*

Trichoderma viride, on rotten grass on wood, *A.*

Verticillium cyclosporum (Grove) Mason & Hughes, on *Fagus*, *D.*

AUTUMN DAY FORAYS, 1954

Our Society has always welcomed visitors on its advertised Day Forays. In response to requests from some of our members, I approached other Natural History Societies to ask permission to advertise their Day Forays in our programme and also permit any of our members who happened to be in their areas to attend the Forays. Permission was freely granted, and I would like to thank the Societies concerned for welcoming any of our members who went to their meetings, which are recorded below.

Home Counties Day Forays organized by the British Mycological Society were held as follows: *18 September*, Windsor Forest; *25 September*, Gomshall, near Dorking; *2 October*, Juniper Hall, near Dorking, 1st area; *9 October*, Virginia Water; *16 October* (joint Foray with South London Botanical Society), Oxshott, Surrey; *23 October*, Juniper Hall, 2nd area; *30 October*, Ashridge Park, Herts.

An outstanding event of these Day Forays was the finding, on 23 October at Juniper Hall, of three toadstools unrecorded for Britain: *Hygrophorus hymenocephalus* Smith & Hesler, first described from North America in 1942; *H. quietus* Kühner, formerly confused with other species, notably *H. obrusseus*, but clearly typified by Prof. Kühner in 1947 from French material and, finally, *Pholiota albocrenulata* Peck. This was first described from North America in 1873 and this is only the third occasion on which it has been recorded in Europe. Not only are these species interesting as new records but also in themselves, and a full account of them is being prepared.

In other provincial areas and in Scotland, the following Day Forays were advertised in our programme as indicated above: *25 September*, Botanical Society of Edinburgh at Tynninghame, North Berwick; North Western Naturalists' Union at Cotterill Clough, near Ringway, Cheshire; Haslemere Natural History Society at Waggoners Wells, Surrey; *26 September*, Botanical Society of Edinburgh at Gifford, East Lothian; Bedfordshire Natural History Society and Field Club at Turvey; *3 October*, Sorby Natural History Society in Hope Valley area; *9 October*, Birmingham University Botany Department at Lickey Hills; Lincolnshire Naturalists' Union at Canwick Park; Sheffield University Botany Department at Ecclesall Woods and Limb Valley; *10 October*, Sheffield University Botany Department at Chatsworth Park; Essex Field Club at Epping Forest; *17 October*, Birmingham University Botany Department at Wyre Forest, near Bewdley; *24 October*, Birmingham University Botany Department in Birmingham area; Northamptonshire Natural History Society and Field Club at Harlestone Firs; Yorkshire Naturalists' Union in Kirkburton district, near Huddersfield.

F. B. Hora
Foray Secretary

THE TWENTY-EIGHTH ANNUAL PLANT PATHOLOGY FIELD DAY

25 June 1954

The Twenty-eighth Annual Plant Pathology Field Day was held at the National Institute of Agricultural Botany, Cambridge. The Director, Mr F. R. Horne, welcomed about 70 members. The party was divided into two, one group touring the Trial Grounds in the morning and the Official Seed Testing Station and Glasshouses in the afternoon, the second group adopting the reverse procedure.

In the Trial Grounds, the following demonstrated aspects of the Institute's work: Mr E. G. Thompson, Head of Crop Improvement Branch, trials with particular reference to cereals; Mr R. S. L. Jeater, the effects of 2-4-D sprays on grass seed crops; Mr R. P. Hawkins, classification of herbage crops and grass strain testing; Dr C. C. V. Batts, variety testing for disease susceptibility, particularly loose smut of wheat and barley; Mr G. C. Hervey-Murray, the Field Approval Scheme for cereals.

In the Official Seed Testing Station, Dr P. S. Wellington (Chief Officer) conducted members around the various sections (grasses, clovers, cereals, and vegetables) and spoke briefly about his own work on premature sprouting in wheat varieties; Dr G. M. Ward demonstrated her work on seed-borne diseases.

In the Glasshouses, Mrs R. S. L. Jeater spoke about the testing of wheat varieties for yellow rust susceptibility and Mr D. Richardson outlined the work of the Potato Branch with particular reference to virus testing.

Members lunched together in one of the city's restaurants. At the end of the afternoon members were entertained to tea by the Institute; and the Chairman of the Plant Pathology Committee, Dr Hickman, thanked the Director and his staff for the interesting day provided for members.

C. C. V. Batts
Secretary, Plant Pathology Committee

THE BRITISH MYCOLOGICAL SOCIETY
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REPRINTS AND MISCELLANEOUS PUBLICATIONS

Plant Pathology Committee: *List of Common British Plant Diseases*, 3rd Ed. 1945. Price net, 5*s*. Postage, 4*d*.

A. A. Pearson: *Cooke's illustrations of British Fungi* (*Annotations by L. Quélet, R. Maire & C. Rea*). *Ex* Vol. 20, Pt. 1. 1935. Price net, 2*s*. 6*d*. Postage, 3*d*.

T. Petch: *British Hypocreales. Ex* Vol. 21, Pts. 3 & 4. 1938. Price net, 2*s*. Postage, 3*d*.

A. A. Pearson & R. W. G. Dennis: *Revised list of British Agarics and Boleti. Ex* Vol. 31, Pts. 3 & 4. 1948. Price net, 5*s*. Postage, 3*d*.

J. Ramsbottom & F. L. Balfour-Browne: *List of Discomycetes recorded from the British Isles. Ex* Vol. 34, Pt. 1. 1951. Price net, 5*s*. Postage, 3*d*.

Fifty Year Index to The Transactions, Vols. 1–30; 1896–1946. Compiled by G. R. Bisby. Price net, 21*s*. Postage, 6*d*.

E. M. Blackwell: Haustoria of *Phytophthora infestans* and some other species. *Ex* Vol. 36, Pt. 2. 1953. Price 4*s*. Postage, 3*d*.

K. Sampson & J. H. Western: *Diseases of British Grasses and Herbage Legumes.* 2nd Edition. 1954. Price, 15*s*. Postage, 6*d*.

Hilda M. Canter: *Annotated List of British Aquatic Chytrids. Ex* Vol. 36, No. 4. 1953. Price 3*s*. Postage, 2*d*.

Malcolm Wilson & G. R. Bisby: *List of British Uredinales. Ex* Vol. 37, No. 1. 1954. Price 3*s*. Postage, 2*d*.

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FORAY COMMITTEE PUBLICATIONS

(*To be obtained from the Treasurer and on sale at meetings and forays*)

A key to the genera of the agarics and Boleti. By A. A. Pearson. 32 pp. 1950. Price net, 2*s*. 6*d*. Postage, 2*d*.

Guide to the literature for the identification of British fungi. 1952. Price net, 2*s*. 6*d*. Postage, 2*d*.

Census Catalogue of British Lichens. By W. Watson. 1953. Price 7*s*. 6*d*.

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INSTRUCTIONS TO AUTHORS

Contributors are asked to be clear and concise, and to use a recent issue of the *Transactions* as a guide to the arrangement of headings and subheadings, explanations of plates and figures, references to the literature, and other details. Each paper should start with a short summary. Contributors are urged not to adopt long serial titles and sub-titles.

The standards adopted in the *Transactions* are those outlined in the Royal Society's pamphlet, *General Notes on the Preparation of Scientific Papers* (1950, Cambridge University Press, 200 Euston Road, London, N.W. 1, price 2*s.* 6*d.*). An exception is that in the *Transactions* citations of references to literature, although based on the Harvard system, include the full titles of the papers and complete page numbers. Titles of journals are abbreviated in accordance with *A World List of Scientific Periodicals*, 1952.

The scientific names of genera and species are printed in italics and should be underlined in the typescript. The title of the paper, headings, and subheadings may be in capitals, but should not be underlined.

The attention of authors proposing new taxonomic groups is drawn to the need to give adequate descriptions and figures, and to designate the types as required by the *International Code of Botanical Nomenclature*. The description of a new species should be accompanied by a figure, and whenever possible the type specimen should be deposited in one of the national herbaria.

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